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Early detection of health disorders in dairy cows based on changes in feeding behaviour

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Introduction Health problems in dairy cows are detrimental to animal welfare, cause production losses and lead to treatment costs. Early detection of health disorders can have cow welfare as well as economic benefits. For disorders that affect short-term feeding behaviour, the automatic monitoring of such behaviour by electronic tagging of cows could assist in early detection. The first objective of the study was to describe and quantify changes in short-term feeding behaviour of dairy cows that occur with the onset of the health disorders ketosis, mastitis, acute locomotory problems and chronic lameness. The evaluation of the suitability of an algorithm based on those changes as a diagnostic tool for the early identification of health problems in group-housed dairy cows was our second objective.

Materials and methods Number of daily visits to computerised feeders, feed intake (FI, as fresh matter, FM) and feeding time (FT) were recorded from group-housed dairy cows in a number of experiments over several years at Langhill Dairy Farm. Cows received 0.5 kg concentrate during each milking and had *ad libitum* access to feeders that supplied mixed rations based on grass silage with, on a FM basis, between 8 and 13% concentrates (high-forage) or between 22 and 30% concentrates (high-concentrate). Feeding behaviour data were related to a Langhill data base containing health records to identify cows for analysis. Cows were excluded from the analysis if they changed feeding treatment or had another disorder during the relevant period. Cows diagnosed with ketosis (n=8), mastitis (n=30) and acute locomotory problems (n=14) were identified and their feeding behaviour before, during and after diagnosis was analysed by linear regression. The feeding behaviour of cows identified with chronic lameness during routine claw trimming (n=81) was analysed by linear regression for 30 d before and 30 d after diagnosis and compared with that of non-lame cows (n=62) that were trimmed at the same time. Across disorders, FT was found to be the best indicator of health problems. An algorithm comparing actual daily FT with the rolling previous 7-d average and standard deviation (SD) of FT was, therefore, tested for its suitability to detect ketosis and acute lameness early.

Results Ketosis was characterized by rapid daily decreases in FI (-10.4 kg/d; $P < 0.01$), FT (-45.5 min/d; $P < 0.01$) and feeding rate (FR, -25.3 g/min; $P < 0.1$) during an average of 3.7 d before diagnosis by farm staff. Mastitis generally increased the variability of feeding behaviour but no characteristic could be identified that should assist in early detection. Cows with acute locomotion disorders showed smaller daily decreases in FI (-1.57 kg/d; $P < 0.05$) and FT (-19.1 min/d; $P < 0.01$) and a daily increase in FR (+21.6 g/min; $P < 0.001$) during an average of 7.7 d from onset to diagnosis. During the 30 d before trimming, cows later classified as lame showed a decrease ($P < 0.001$) in daily FT and an increase ($P < 0.001$) in FR but non-lame cows did not. These changes reversed after trimming (Figure 1). The changes in feeding behaviour were not different between cows consuming high-concentrate or high-forage diets. An algorithm that identified cows with a daily FT lower than the previous 7-day rolling average minus 2.5 SD resulted in detection of more than 80% of cows with ketosis and acute lameness between one and eight d earlier than diagnosis by farm staff.

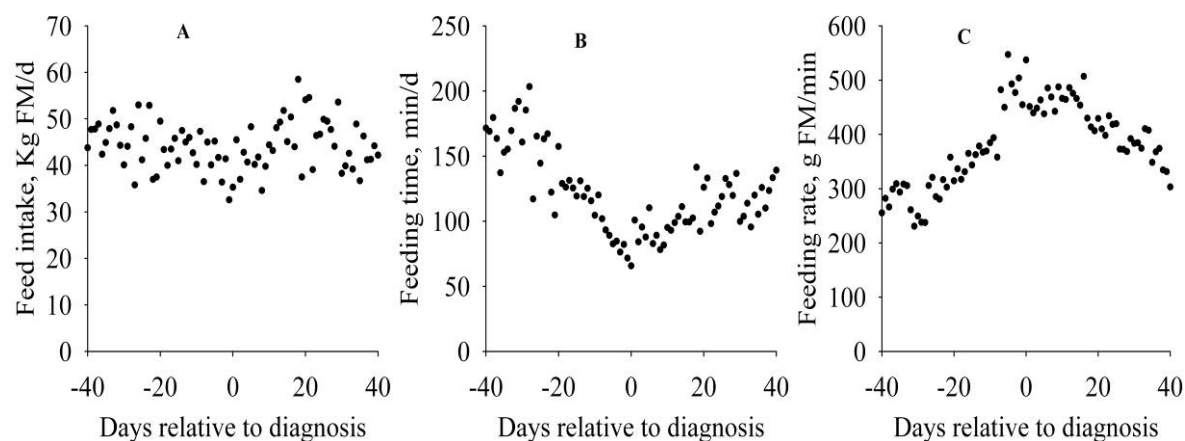


Figure 1 Example of characteristic changes in daily feeding time (B) and feeding rate (C) but not in daily feed intake (A) associated with chronic cases of lameness that were detected at the time of claw trimming (day 0). Trends in feeding time and feeding rate of the same magnitude as shown were observed stretched out over periods up to 100 days in chronic cases of lameness or compressed into one week before diagnosis in acute cases of lameness.

Conclusions We found no evidence that mastitis results in any systematic changes in feeding behaviour that can assist in its detection. Short-term feeding behaviour showed very characteristic changes, however, with the onset of ketosis and acute lameness, and similar, but more gradual, changes during chronic lameness. The work shows that a monitoring system with an algorithm that analyses feeding time, which does not require (expensive) intake measurements, can be very useful for the early detection of cows with such disorders.

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Relationship between lameness and lying behaviour of zero-grazed Holstein dairy cattle recorded using an activity monitor

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Introduction Lameness can be assessed using locomotion scoring; however, this method is time consuming and an automated method of detecting lameness is needed. Studies have shown that in conventionally-managed dairy herds, lame cows spend more time lying down than non-lame cows (e.g. Singh *et al*, 1993). However, there are limited data available for high producing zero-grazed dairy cattle in the UK. IceTags™ are activity monitors which use an electronic accelerometer to determine the percent of time spent standing, lying or active and have been validated in a study by Munksgaard *et al* (2006). The aim of the present study was to assess the impact of lameness on lying behaviour of high yielding, zero-grazed, Holstein dairy cows and to assess the potential use of lying time to detect lameness.

Materials and methods The study was conducted on a commercial 500 cow, zero grazing dairy herd in the south east of the UK from 15th Jan to 2nd Feb 2007 using 59 lactating Holstein dairy cows (11 primiparous and 48 multiparous). The cows were housed all year round in a free stall cubicle yard; on mats bedded with sawdust. Cattle were milked three times daily throughout the study. The lameness of the cows was assessed using the locomotion scoring method (locomotion score 1-5; score 1 normal gait; score 5 severe lameness) of Flower and Weary (2006). No cows scoring 4 or 5 were used in the study as there were too few animals of this score. For analysis cows were grouped according to locomotion score (1, 2, 3). Groups were balanced for stage of lactation and parity (parity 2.3 ± 0.10 ; 174 ± 8.8 DIM (mean \pm SEM)). Immediately after locomotion scoring, activity monitors (IceTag™, Ice Robotics Ltd, Roslin, UK) were attached to the back right leg above the fetlock for 4 days to measure standing and lying behaviour. Data from the IceTags were used to determine the percentage of time that the animal spent standing, lying or active; the data were converted to hours/day. Lying bouts of less than 2 minutes were discarded as likely to be incidences where the cow lifted the leg on which the IceTag™ was attached. The mean lying bout time was calculated as the number of consecutive minutes that the proportion of time spent lying was recorded as being greater than 96%. The minimum and maximum duration of lying bouts was calculated from the mean of 4 days data. Frequency of lying was determined as the mean number of times a cow lay down in a period of 24 hours. Daily milk yield was measured over the week of study.

All data were normally distributed (using Pearson's skewness test) and differences between groups were assessed by one-way Analysis of Variance using Genstat (Version 8, Lawes Agricultural Trust).

Results Cows with locomotion score 3 spent approximately 2 hours/day longer lying down ($p=0.008$) than cows with locomotion score 1 and locomotion score 2. There was no effect of locomotion score on time spent active. Locomotion score 3 cows had longer mean lying bouts ($p=0.13$) and maximum lying bout ($p=0.11$) than cows with locomotion score 1 and 2

Locomotion score 3 cows produced 8.0 and 6.4 litres ($p=0.03$) less milk compared with cows with locomotion score 1 and 2, respectively.

Table 1 The association between locomotion score on the activity and milk production of dairy cattle

Parameter	Locomotion Score			s.e.d.	Significance
	1 (n=16)	2 (n=21)	3 (n=22)		
Lying Down (h/day)	10.9 ^a	11.1 ^a	13.0 ^c	0.74	*
Standing (h/day)	12.2 ^a	12.0 ^a	10.2 ^c	0.69	*
Active (h/day)	0.9	0.9	0.8	0.08	NS
Lying Bouts per day	11.1	9.8	10.9	1.17	NS
Mean Duration of Lying Bout (min)	60.7	69.0	76.2	7.44	NS
Minimum Duration Lying Bout (min)	6.4	6.9	8.4	2.22	NS
Maximum Duration Lying Bout (min)	151.4	168.9	190.9	18.41	NS
Milk Yield (l/day)	37.3 ^a	35.7 ^{ab}	29.3 ^b	3.19	*

^{a,b}. Means with different superscripts are significantly different ($p<0.05$)

Conclusions The results demonstrate that lying time measured using an activity monitor has the potential to detect lameness in dairy cattle as indicated by increased lying times. These findings could contribute to the automation of lameness detection. Further work is required to assess the causes of impaired locomotion and their relationship with lying behaviour.

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Influence of post-calving regrouping strategy on the behaviour and performance of dairy heifers

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Introduction The integration of heifers with the main dairy herd during the post calving period can lead to reduced welfare and production performance. The aim of this study was to assess whether any benefits to welfare or productivity are shown when heifers are introduced to groups of mature cows as pairs rather than individuals. In addition, the benefit of allowing heifers a 1-week recovery period after calving before being introduced to these groups was also assessed.

Materials and methods Thirty-six Holstein Friesian heifers (Predicted Transmitting Ability (PTA₂₀₀₅) Fat + Protein yield of 29.4 (s.d. 8.1) kg) were assigned to one of three treatments over six replicates. The treatments were as follows: (1) Heifers introduced individually to an established group within 1 day of calving ('S1'), (2) Heifers kept individually in a straw pen for 7 days post calving before being added individually to an established group ('S7'), (3) Heifers kept as a pair in a straw pen for 7 days post calving before being added as a pair to an established group ('P7'). Two heifers were assigned to each treatment per replicate. Two weeks prior to the start of each replicate a resident group was established which contained 10 mature cows and 6 'non-experimental' first lactation heifers. These 'non-experimental' heifers were removed from the group prior to experimental heifers being added. The resident group was housed in a cubicle pen, and all heifers had experience using cubicles. The resident group was video-recorded continuously during days 1, 2, 3 and 5 of the first week following addition of experimental heifers. The group was also recorded for 2 x 24 hour periods during weeks 2, 3 and 4 after heifers were added. Instantaneous scans were made of each of the newly-introduced heifers at 15 minute intervals for the first 8 hours and at hourly intervals in all further observations. The location of the heifers and their state (lying or standing) were recorded during these scans. Whether or not more than half the entire group was lying during these observations was also recorded to assess synchrony of lying behaviour. Milk yield was recorded daily from 2 to 28 days post calving, and milk composition was assessed from one morning and afternoon (bulked) sample per week. Location and state behavioural data were analysed by repeated measures analysis of variance (ANOVA), and milk production data by ANOVA (using PTA₂₀₀₅ values as covariates). A 'z' test was carried out to assess if proportions of scans where 'P7' heifers were in the same pen area or in adjacent cubicles (expressed as a proportion of scans, or as a proportion of scans when heifers were in cubicles, respectively) was greater than zero. A Fisher's exact test was also carried out to assess whether these heifers fed independently at the silage face. This was to ensure that evidence of heifers spending significant amounts of time in the same pen area did not simply reflect synchronicity of silage feeding.

Results Heifers in the 'P7' treatment spent more time than expected in the same pen area during the first 8 hours in the group (proportion of scans 0.25, z value 4.332, P<0.001), and during the first month in the group (proportion of scans 0.21, z-value 11.410, P<0.001). These heifers also spent more time than expected in adjacent cubicles during the first month in the group (proportion of scans in cubicles 0.23, z-value 10.25, P<0.001), but not during the first 8 hours in the group (P>0.05). The Fisher's exact test for independence of silage feeding behaviour was not significant during the first 8 hours in the group (P>0.05), but was significant for the first month in the group (P<0.001).

Selected results are presented in Table 1. Heifers in the 'P7' treatment tended to spend a lower proportion of time standing (when more than half the group were lying) than heifers in other treatments during the first 8 hours in the group (P<0.06). During the first month in the group heifers in the 'P7' treatment spent more time standing than heifers in other treatments (P<0.05), however there were no significant differences between treatments in the time spent standing when more than half of the group was lying (P>0.05). Heifers in the 'P7' treatment also spent less time in cubicles during the first month in the resident group than heifers in the 'S7' treatment, with those in the 'S1' treatment being intermediate (P = 0.05). Finally, heifers in the 'P7' treatment tended to show greater protein yields, and fat plus protein yields during the first month post calving than heifers in other treatments (P<0.1).

Table 1 Influence of post-calving regrouping strategy on behaviour and performance parameters in dairy heifers

	Single – day 1	Single – day 7	Pair – day 7	SEM	P
Standing up (> half the group lying) (First 8 hours)	0.19	0.21	0.12	0.025	<0.06
Standing up (First 8 hours)	0.89	0.87	0.92	0.038	NS
Standing up (> half the group lying) (First month)	0.17	0.16	0.18	0.012	NS
Standing up (First month)	0.61 ^a	0.59 ^a	0.67 ^b	0.020	<0.05
In cubicles (First month)	0.60 ^{ab}	0.62 ^b	0.57 ^a	0.015	0.05
Milk fat plus protein yield (kg/day)	1.88	1.78	1.98	0.060	<0.1

^{a,b} Means in the same row with different superscripts differ significantly, P<0.05.

Conclusions Housing heifers as pairs for 1 week prior to introduction to the resident group appeared to promote association between the heifers within the group. These heifers also appeared to show more synchronous lying behaviour in the immediate post mixing period, and increased activity during the first month post calving. These factors may have reflected reduced fearfulness. Allowing a 1-week 'recovery' period after calving did not appear to improve welfare or productivity.

There is no evidence of impairment of reproductive function of dairy cows on spring pasture during mating in New Zealand (NZ)

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Introduction Highly degradable protein diets creating high ammonia and/or urea concentrations in blood or reproductive fluids may affect reproductive performance by their toxicity to embryos (Hammon *et al.* 2005). Pastures in NZ during spring have high CP (170 to 273 g/kg DM) and cows on these pastures have high blood urea (36 mg/dl; Moller *et al.* 1993). Few studies (Ordonez *et al.* 2007) have evaluated association between high blood urea and reproduction of individual cows. The present study examined individual cow reproductive performance and blood urea concentration during grazing of spring pasture.

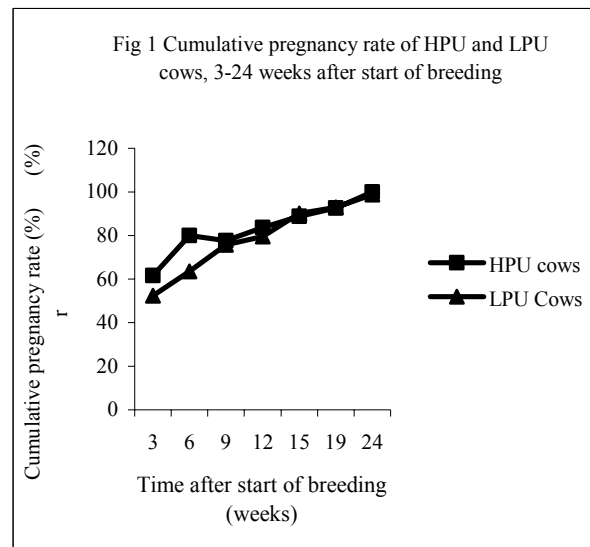
Materials and methods 200 cows/week were bled during a six week breeding period (n = 1189 samples) from a herd of 528 cows. A subset (n = 211) of cows with a blood urea concentration measured within ± 10 days of insemination were analysed. The herd experienced routine commercial pasture management. Plasma urea concentrations were determined on a Cobas Mira Plus autoanalyser. Dates on when cows were inseminated, pregnancy diagnosed, subsequently calved, and previous calving date were retrieved from herd data. The effects of plasma urea status (high, HPU and low, LPU, relative to the sample mean; 44.9 mg/dl) on submission rate (SR), non-return rate (NRR), and calving rate (CR) were tested using Chi-square analysis. The effects of plasma urea status on plasma urea concentration, number of services (NS), interval between first and second AI (IFSAI), interval from calving to conception (ICC), interval from calving to first service (ICFAI) and calving interval (CI) were evaluated using GLM procedure.

Results Reproductive performance of sampled cows was similar to that of the whole herd, indicating that they were representative of the herd. HPU cows had a high ($P < 0.001$) blood urea concentration (50.8 ± 1.1 vs 38.5 ± 1.1 mg/dl) compared with LPU cows. There was no difference ($P > 0.05$) in NRR and intervals between reproductive events between the groups (Table 1). Pregnancy rates from 3 to 24 weeks after breeding were similar ($P > 0.05$) except at 6 weeks when they were higher ($P < 0.05$) for HPU than LPU cows (Fig 1).

Table 1. Reproductive performance of cows on spring pasture

Parameter	N	HPU	LPU	P-value
Urea (mg/dl)	211	50.8 \pm 1.1	38.5 \pm 1.1	<0.0001
SR (%)	211	85.0	77.4	0.16
NS ¹	211	1.53 \pm 0.1	1.55 \pm 0.1	0.83
NRR (%)	211	53.0	41.4	0.09
ICC (d)	137	86.1 \pm 1.8	87.8 \pm 1.9	0.52
IFSAI (d)	92	23.9 \pm 1.3	22.6 \pm 1.1	0.46
Gestation (d)	189	276 \pm 1.8	272 \pm 1.7	0.09
CI (d)	137	368 \pm 2.0	370 \pm 2.2	0.60
ICFAI (d)	137	78.0 \pm 1.0	79.2 \pm 1.1	0.41
CR (%)	211	98.0	97.3	0.78

¹No of services = includes natural breeding,



Conclusions Lack of difference in reproductive traits of the two groups may be due to: 1) both the HPU and the LPU animals may have been affected to the same extent since the LPU had plasma urea concentrations similar to a value of 42.9 mg/dl, above which Ferguson *et al.* (1993) found to affect conception rate in dairy cows. 2) Farmers in NZ have selected cows for good reproductive performance within a high protein pasture environment and hence cows may have developed resistance to any detrimental effect of ammonia.

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Relationship between knowledge factors and lameness levels on dairy farms in Northern Ireland

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Introduction Lameness is a significant problem leading to reduced dairy cow productivity and welfare. Housing and management factors are known to influence lameness levels in dairy cows (Haskell *et al.*, 2006). Furthermore, lameness management strategies are also key factors affecting the incidence of lameness (e.g. early detection of lameness (Whay, 2002) and claw trimming (Manske *et al.*, 2002)). It is likely that knowledge factors influence lameness management strategies (Mill and Ward, 1994). The current study aimed to gain a better understanding of the relationship between lameness knowledge levels and the incidence of lameness on-farm. This is part of a wider survey aimed at understanding how knowledge, attitude, perception, and management factors affect lameness levels on dairy farms.

Materials and methods Fifty-nine dairy herds were visited during the winter housing periods of 2005/6 and 2006/7. The survey included farms throughout Northern Ireland. Average milking herd size was 136 ± 53 (Mean \pm SD) cows. A one hour interview with a set questionnaire was carried out with the person who made the majority of lameness management decisions. The present study focused on the lameness knowledge aspect of the larger survey data set.

Lameness knowledge was assessed by asking producers to identify common conditions leading to lameness from photographs and to suggest possible causes, and treatments. The eight photographs included overgrown claws, interdigital phlegmon ("foul"), heel erosion, swollen hock, sole ulcers, white line abscesses, interdigital hyperplasia, and digital dermatitis. Producer responses were scored as 'correct', 'partially correct', or 'incorrect'. An 'overall knowledge' score was calculated as the proportion of correct/partially correct answers to all 24 questions.

The milking herd was locomotion scored using the Flower and Weary (2006) scoring system. A score of 1 = sound cow (with smooth, fluid movements) and 5 = severely lame cow. Any animal scored 3 or higher was considered clinically lame (score 3 = slight limp detected). Locomotion scores were analysed in three different categories: (1) locomotion score 3 and higher ('Lame 3'); (2) locomotion score 3.5 or higher ('Lame 3.5'); and (3) locomotion score 4 or higher ('Lame 4'). Data were analysed using generalized linear mixed models and binomial logistic regressions in GenStat.

Results On average, producers correctly identified 85.38% of the conditions from photographs (minimum: 50% correct; maximum: 100% correct). Producers were able to identify common conditions such as overgrowth, swollen hock, sole ulcers, white line abscesses, and digital dermatitis more easily than interdigital hyperplasia, interdigital phlegmon ("foul"), and heel erosion. A similar pattern was seen in the responses for the cause and treatment of these conditions.

Overall knowledge was related to the number of severely lame cows on-farm ('Lame 4') (OR = 0.06; 95 % CI = 0.004 - 0.86; $P=0.04$). Knowledge of individual lameness issues shown in photographs was not related to the level of lameness in most cases. However, if the producer could suggest (partly or completely) correct causes of claw overgrowth, the odds of having lame cows ('Lame 3') decreased compared to farms where the producer could not suggest correct contributing factors to overgrowth (OR= 0.53; 95% CI= 0.34 - 0.83; $P=0.008$).

Overall knowledge was not related to producer age, level of education, whether the farm was a mixed enterprise (more than just milk sales), herd size, average herd age, or whether or not the producer believed he had a viable future in dairying. However, there was a trend between herd size and overall level of knowledge in relation to lameness. Producers with larger herds tended to have lower overall knowledge scores ($P=0.075$).

Conclusions These results show that dairy producers in Northern Ireland have a high level of knowledge in relation to lameness conditions. Nevertheless, the data show that it is important for dairy producers to stay informed on lameness issues. Producers with larger herds showed a tendency for lower lameness knowledge. There is a continuing need for training to increase knowledge and awareness of lameness in dairy cattle to help producers reduce the incidence of lameness.

Acknowledgements The authors gratefully acknowledge funding from AgriSearch and DARDNI.

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The impact of claw horn lesions and digital dermatitis on dairy cows' performance

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Introduction Foot and leg problems are a major health and welfare concern for the dairy industry. Lameness is believed to be a major economic cost, due to lost performance and premature culling. The aim of this study was to look at the two most prevalent foot problems within the Langhill dairy herd and assess the predisposing factors and its impact on the cow's performance.

Materials and methods Performance indicators included feed intake, milk fat and protein composition, milk yield, fertility and culling. Data were studied from 2914 completed lactations from the Holstein Friesian Langhill Dairy herd between January 1990 and August 2005. The Langhill herd are on a long-term genetic breeding and feeding systems project. Approximately equal numbers of select line (selected for kilograms of fat plus protein) and control line cows (average genetic merit for fat plus protein production) were offered either a high or low concentrate diet as a total mixed ration (TMR). There were 739 (from 515 cows) and 802 cases (from 529 cows) of digital dermatitis (DD) and claw horn lesions (CHL) respectively in the Langhill herd, a level of 25 per cent and 28 per cent for the respective feet problems during the study period. Cows without any diagnosed health problems during their given lactation (healthy cows) were paired on lactation number, genetic group, feed group and farm to cows that were diagnosed with only DD or a CHL during their respective lactation. The herd was managed from 1990 to 2002 at the University of Edinburgh, and then from 2002 at the SAC Dairy Research Centre, both in Scotland. There were 83 cows with DD and 65 cows with CHL that could be paired to a healthy cow. The presence of DD and CHL were diagnosed by the farm veterinarian. For this study, DD describes infection of the skin surrounding the claw (including interdigital growths and foul in the foot) and CHL is the lesions of the claw such as ulcers, white line disease and sole haemorrhage (not including sand cracks and overgrown claw). The data were analysed using a general linear mixed model as described by Breslow and Clayton (1993) in Genstat Version 7.2 (Lawes Agricultural Trust, 2004) with significance being attributed at $P < 0.05$. Using a chi-squared (χ^2) test, the incidence of conception failure and incidence of culling for all cows over the study period were each analysed with the occurrence of DD and CHL to test their association.

Results DD was associated with culling ($\chi^2 = 23.78$, $P < 0.001$) and CHL was associated with conception failure ($\chi^2 = 4.56$, $P < 0.05$). Table 1 highlights the predisposing factors associated with DD and CHL. Table 2 shows the effects of DD and CHL, which shows no impact on the cow's performance in terms of feed intake, milk yield, milk composition.

Table 1 Raw data means (se) from the multivariate analyses showing the predisposing factors associated with DD and CHL

Variable	Wald statistic	df	P	Drying off period liveweight change (kg)			
				<-39	-39 to -15	-14 to 10	>10
Claw horn lesion	10.1	3	<0.05	0.25 (0.05)	0.13 (0.04)	0.10 (0.03)	0.07 (0.02)
				Lactation length pre-calving (days)			
				<296	296 to 316	317 to 347	>347
Digital Dermatitis	14.68	3	<0.01	0.05 (0.02)	0.20 (0.04)	0.14 (0.04)	0.07 (0.03)

Table 2 Raw data means (se) from the multivariate analyses showing the main effects of DD and CHL

Variable	Wald statistic	df	P	Lactation group				
				1	2	3	4	5
Early lactation								
Claw horn lesion	18.87	4	<0.001	0.04 (0.02)	0.07 (0.02)	0.04 (0.03)	0.11 (0.06)	0.22 (0.10)
				Average 1 to 21 DIM milk yield (kg)				
Early lactation				<24.29	24.29 to 30.66	30.67 to 35.66	>35.66	
Claw horn lesion	7.95	3	<0.05	0.05 (0.02)	0.05 (0.02)	0.06 (0.03)	0.18 (0.06)	
				Average 1 to 21 DIM milk fat composition (g/kg)				
Early lactation				<41.21	41.21 to 45.39	45.40 to 49.45	>45.45	
Digital Dermatitis	9.17	3	<0.05	0.06 (0.02)	0.04 (0.02)	0.04 (0.02)	0.14 (0.04)	
				Calving to first service (days)				
Mid lactation				<64	64 to 74	75 to 87	>87	
Digital Dermatitis	12.52	3	<0.01	0.03 (0.02)	0.02 (0.01)	0.10 (0.04)	0.19 (0.06)	
				Average 1 to 200 DIM dry matter intake (kg)				
Late lactation				<17.07	17.07 to 19.29	19.30 to 21.26	>21.26	
Digital Dermatitis	8.57	3	<0.05	0.03 (0.02)	0.03 (0.02)	0.07 (0.03)	0.16 (0.06)	

Conclusion DD has a direct effect on the lifespan of a dairy cow. Both DD and CHL can be reduced by management of the environment in which dairy cows live.

Acknowledgements We are grateful to the farm staff and Ross McGinn for capturing the data.

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The puncture force of hoof horn from New Zealand Friesian and Jersey Cross Friesian dairy cattle breeds

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Introduction Globally up to 60% of dairy cattle can become lame at least once a year (Vermunt and Greenough, 2005). Sole lesions have been found to be one of the most common causes of lameness in both NZ (Tranter and Morris, 1991; Chesterton, 2004) and the UK (Kossabati *et al.*, 1999; Logue, 1999). In most studies it was found that these hoof horn lesions develop in postpartum animals and in hind claws (Greenough and Vermunt, 1991) and that these are associated with a significant reduction in the puncture force of the hoof (Winkler and Margerison, 2003). There has been some anecdotal reference to lower levels of lameness and harder hoof horn occurring in Jersey and Jersey cross bred cattle, compared with Friesian dairy cattle. However no studies have measured or compared the structural strength of hoof horn within these breeds. The aim of this research was to assess the force to puncture of the hoof horn from differing claws and regions of claws in prepartum New Zealand Friesian (F) and Jersey cross Friesian (Jersey x) dairy breeds.

Materials and methods At 14 weeks of age all four hooves from 9 (5 Jersey x; 4 NZ Friesian) male dairy cattle and at 15 months of age 30 female (15 NZ Friesian and 15 Jersey X) had their hooves held and the distal 1mm surface of the sole horn removed. All the claws were scored for the presence of lesions according to Leach *et al.* (1998) and hoof tissue samples were taken from the weight bearing regions 1 to 5 of the international foot map. The hoof samples stored in sealed plastic bags at 4 °C until analysis using a texture analyser and oven dried for dry matter determination (according to Winkler and Margerison, 2003). The data was normalised by log transformation and analysed by ANOVA GLM (Minitab 14.0) individual claw were used as observations and breed, claw, region of the claw were included as nested factors in the model and significant differences were assessed, with a confidence interval of 95%, using Tukey's test and Bonferoni correction was applied.

Results The puncture force (PF) of hoof horn taken from Friesian and Jersey cross breed cattle is presented in Table 1 and 2. The puncture force of hoof tissue from Jersey cross bred was consistently higher for all regions of the foot map compared with Friesian dairy cattle. The PF of hoof horn taken from regions 1 and 2 (white line) had consistently lower puncture force compared with regions 4 and 5 (sole) for both Jersey and Friesian cattle, while the PF of region 3 was intermediate between the white line and sole areas. The PF of hind and fore claws did not differ significantly.

Table 1 Puncture force (log₁₀ N) of hoof horn from Friesian and Jersey cross dairy cattle

Region of foot	Breed		sem	P Value
	Friesian	Jersey cross		
1	0.67 ^c	0.85 ^b	0.021	0.001
2	0.60 ^c	0.70 ^c	0.022	0.001
3	0.73 ^b	0.85 ^{a,b}	0.023	0.001
4	0.78 ^{a,b}	0.91 ^a	0.020	0.001
5	0.84 ^a	0.90 ^a	0.021	0.001
sem	0.013	0.019	-	-

^{a,b,c}Data in the same column followed by differing superscripts differ significantly (P<0.01)

Table 2 Puncture force (log₁₀ N) of 1 and 2 (white line) and 4 and 5 (Sole) areas of the international foot map (IFM)

Puncture force (log ₁₀ N) of:	Breed (B)		Hoof (H)		sem	P value		
	Friesian	Jersey cross	Fore	Hind		Breed	Hoof	B*H
Mean white line (IMF 1 and 2)	0.63 ^b	0.75 ^a	0.71	0.68	0.013	0.001	0.097	0.185
Mean sole (IMF 4 and 5)	0.80 ^b	0.89 ^a	0.84	0.86	0.010	0.001	0.244	0.027

Conclusions Hoof horn with higher PF is more likely to be resilient to challenges of housed and pasture based cattle and result in lower levels of lameness. Jersey cross bred cattle had hoof horn that had a significantly higher puncture force than Friesian cattle. However, the PF of white line tissue was consistently significantly lower than that from the sole regions 4 and 5 making this area less resilient in both breeds. The PF of tissue from fore and hind claws did not differ significantly, which indicates that lower puncture resistance of hind claws, occurs later in life.

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Production responses to temperature and humidity index in lactating dairy cows

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Introduction One of the greatest challenges to production facing dairy farms in the north and north-east of Iran is heat stress. Climatic conditions in this area are such that warm (or hot) season is relatively long, as generally there is high relative humidity. Lactating dairy cows produce a huge quantity of metabolic heat. Heat produced and accumulated in the body of dairy cows. The body temperature rises, intake declined and finally the cow's productivity reduce as temperature-humidity index (THI) rise up. The purpose of this study was to determine the effect of THI on milk production and constituents in the north-east of Iran.

Materials and methods Average milk production and composition data were obtained from a large dairy herd with 500 lactating cows during 2001 to 2006. Annual average milk yield was similar (28-28.5) and culling rate was 18-20% during the study. No attempt was made to account for production change that were due to freshening cycles or age of the herd or for yearly fluctuations in normal production levels. The reproductive program was nearly similar and done by the same practitioner group. Diets were balanced to meet NRC 2001 requirements in various lactating groups of cows. Temperature and humidity percentage was collected from a weather station located 1 Km far from the dairy unit. THI was calculated using $THI = td - (0.55 - 0.55RH)(td - 58)$ where td = dry bulb temperature, RH = relative humidity expressed as a decimal. Data were analysed in a factorial experiment based on a completely randomized design using GLM procedure of SAS 9.1 and means were compared using Duncan test ($P < 0.05$).

Results As can be seen in Table 1 during 2001-2006 there is no changes in maximum temperature of the climate in north-east of Iran but humidity increased about 6 to 8 percent after 2001 ($P < 0.05$). THI was equal at the years of this study. There is a huge increase in daily milk production from 2001-2002 to 2003-2006 ($P < 0.05$) which is related to some changes in nutrition and other management methods used in the herd. Data showed that this increase in milk production had no adverse effect on fat percentage. As can be seen in 2005 and 2006 the fat percent is equal with 2001. The average milk yield was 16.13 Kg so FCM was increase significantly during these years ($P < 0.05$). When changes in climate and productive parameters in different seasons reviewed, it was concluded that there is a correlation between climatic and productive parameters. Table 1 show that in north-east of Iran the maximum temperature and lowest humidity and the maximum temperature humidity index (THI) takes place in summer ($P < 0.05$). Moderate risk of heat stress which had mentioned in literature may happen at this situation (Armstrong, 1994; Armstrong *et al.*, 1999). Daily milk yield is lowest at summer because of the high THI and in case of reducing THI in spring, autumn and winter respectively milk yield begins to increase and the most daily milk takes place at winter ($P < 0.05$). An important matter is that there is a significant low fat percentage ($P < 0.05$) in addition to low milk production at summer so that FCM4% show most difference between hot and cold seasons ($P < 0.05$).

Table 1 Yearly and seasonal changes of climatic and productive parameters in north-east of Iran

Items	Years						SEM
	2001	2002	2003	2004	2005	2006	
Max Temperature (°C)	24.81	23.34	23.71	23.56	23.34	23.71	0.487
Humidity (%)	60.05 ^b	66.31 ^a	68.04 ^a	67.29 ^a	66.30 ^a	68.04 ^a	1.256
THI2	71.40	69.85	70.72	70.59	69.84	70.70	0.646
Daily Milk (Kg)	16.13 ^c	17.03 ^c	21.45 ^b	28.26 ^a	28.22 ^a	27.93 ^a	0.345
Fat (%)	3.42 ^{ab}	3.44 ^a	3.31 ^c	3.16 ^d	3.35 ^{bc}	3.44 ^{ab}	0.030
FCM4%	14.74 ^c	15.61 ^d	19.24 ^c	24.71 ^b	25.49 ^{ab}	25.59 ^a	0.292
	Seasons				SEM		
	Spring	Summer	Autumn	Winter			
Max Temperature (°C)	25.41 ^b	33.78 ^a	21.75 ^c	13.94 ^d	0.397		
Humidity (%)	62.83 ^c	56.21 ^d	68.49 ^b	76.49 ^a	1.025		
THI2	72.74 ^b	84.13 ^a	68.33 ^c	56.88 ^d	0.527		
Daily Milk (Kg)	23.74 ^b	19.98 ^c	23.20 ^b	25.76 ^a	0.282		
Fat (%)	3.34 ^b	3.25 ^c	3.36 ^b	3.45 ^a	0.025		
FCM4%	21.34 ^b	17.71 ^c	20.98 ^b	23.56 ^a	0.238		

Items with different letter in each row have significant difference ($P < 0.05$)

Conclusion It seems that there is a severe reduction in both milk production and fat% of Holstein cows in north-east of Iran and some considerations must be taken to reduce its side effects on economic output of herds in this region at Iran.

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Culling reasons relationship with milk yield, type and parity of Iranian Holstein cows

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Introduction Culling is the act of identifying and removing a cow from a herd and, assuming a constant or expanding herd size, replacing the cow with another cow, often a first-lactation heifer. The culling rate describes the percentage of cows removed from a herd. Understanding culling rates is important for managing dairy production response and profitability. When dairy farm managers cull cattle too often or too quickly, replacement expenditures are excessive. When managers keep cattle for too long, milk production, reproduction, or genetic improvement may be impaired. Past research has consistently estimated optimal herd-level culling rates ranging from 19 to 29%. Researches found that poor reproduction rate was the primary reason for culling, followed by low production and mastitis. Higher milk producing herds were more likely to cull a cow for abortion and reproduction, but less likely to cull for mastitis. So the objective of this study was to investigate the relationships between different reasons of culling and age at time of culling, mean type score of cows, parity at the time of culling, total milk production at 1st parity.

Materials and methods In order to investigate relationship of culling with production and type of cows, 5 herds sized about 100-500 dairy cattle between years 2001-2006 were selected and data was recorded using a computerized record keeping method. Data (355 cows) was selected from 650 records. Monthly milk production was recorded and total milk production (305 days, 3X) was calculated by National Animal Breeding Organization. Type scores were determined in 1st and 2nd parities by a specific judging person and the mean scores were used at this study. Management of the herds was similar and cow's diet was calculated to meet NRC 2001 and was fed to all cows as TMR. Cows were housed in an open shed system using free stalls. Data were analysed based on a completely randomized design using GLM procedure of SAS 9.1. Means were compared using Duncan test ($P < 0.05$).

Results As can be seen in Table 1 the lowest age at culling was in the group of cows which were culled because of mastitis disease ($P < 0.05$). Cows omitted from the herd because of less economic profit or low production, were the oldest age group ($P < 0.05$). The same cows had least mean type score ($P < 0.05$). Cows that are culled because of metabolic diseases had highest parity at the time of culling after economic culls as a reason for cows exiting the herds ($P < 0.05$). Uneconomic cows had least total milk production ($P < 0.05$). Cows culled with reproductive problems had produced the most milk just like metabolically and mastitis based culls ($P < 0.05$). The most reason of cows culling in the herds was reproductive problems which are followed by metabolic problems (105 and 90 cows respectively). Mastitis and economic reasons are equal third reason of culling.

Table 1 Some traits of cows which were culled from herds for the following reasons

Items	Culling Reasons						SEM
	1	2	3	4	5	6	
Age at cull (Day)	2128 ^b	2400 ^{ab}	2278 ^{ab}	1991 ^b	2028 ^b	2596 ^a	45.36
Mean type score	78.85 ^{ab}	79.33 ^a	78.59 ^{ab}	77.86 ^{ab}	79.88 ^a	76.80 ^b	0.245
Parity at cull	3.73 ^b	3.60 ^b	3.76 ^b	3.33 ^b	3.19 ^b	5.03 ^a	0.104
Milk at 1 st parity	7083 ^a	6892 ^{ab}	6957 ^{ab}	6944 ^{ab}	7494 ^a	6273 ^b	80.50

Items with different letter in each row have significant difference ($P < 0.05$).

- 1- Internal disease
 2- Reproductive disease
 3- Metabolic disease
 4- Mastitis
 5- Suddenly culls
 6- Economic culls

Conclusion Results showed that culling data which are kept on herds needed to be more controlled or recorded carefully. Mean production of first lactation cows is about 7000 Kg and reproduction, metabolic and mastitis are the main reason of culled cows. Therefore more specific herd management is needed due to genetic improvement and high milk production to increase productive life of cows from the current position (3.19 to 3.73 in varying culling groups). Cows which continue their productive life up to 5th parity produced more milk but had less type score and it seems that more attention to type parameters in these cows is essential because they put more daughters in herd and generate there genes more efficiently.

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Agewean – The effect of weaning age on growing pig health and performance in the absence of antibiotic growth promoters

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Introduction The immediate postweaning period in pigs is often characterised by a reduced and variable food intake, digestive disorders and poor growth and development. Historically such effects were reduced by the use of in-feed antibiotic growth promoters (AGPs), copper sulphate and zinc oxide to enhance the efficiency of feed conversion and hence maximise nutrient capture. However from January 2006 the routine use of in-feed AGPs was banned and, due to concern over environmental pollution, levels of inclusion of heavy metals are limited by regulation and likely to be further reduced in the future. Weaning pigs at a later age has been suggested as an approach to reduce the potentially negative effects of the AGP ban on the national herd. The objective of the AGEWEAN programme of research was to investigate the effects of weaning age (4, 6 and 8 weeks) in both an indoor and outdoor lactation environment on the biological and economic efficiency of production where diets contain no AGPs and lower levels of copper (<25ppm added) and zinc (<100ppm added).

Materials and methods The research was carried out at 6 separate experimental sites, chosen to represent a range of diverse geographical locations and production systems within the UK. Two sites were used to provide an outdoor lactation environment (total of 100 sows per weaning age treatment) and four sites to provide the indoor environment (total of 90 sows per treatment). The three weaning age treatments were 4 weeks (21–28 days of age), 6 weeks (35–42 days of age) and 8 weeks (49–56 days of age). On any given site gilts were introduced at the point of farrowing and were followed through four consecutive parities (Edge *et al.*, 2007). All progeny were monitored to weaning, at least 50% were monitored to 30kg live weight and at least 25% were monitored through to slaughter weight. The statistical significance of the weaning age effects was assessed using REML analyses (in Genstat), allowing residual variances to differ between sites, treating site as a random effect, and parity and weaning age as fixed effects.

Results There were significant differences between the three weaning age treatments in terms of DLWG, feed intake and FCR during the immediate postweaning period. Pigs weaned at 8 weeks of age had significantly higher DLWG and feed intakes. However their feed conversion efficiency was poorer, possibly due to them being offered a lower quality diet appropriate to their age. Conversely, from 30kg to the point of slaughter pigs weaned at 4 weeks of age had significantly higher DLWG and feed intake than pigs weaned at either 6 or 8 weeks of age. There was no significant difference in feed conversion efficiency (by 30kg all pigs were offered the same specification diet). There were significant effects of weaning age on the back fat thickness (P2 mm) of the pigs at the point of slaughter. In terms of overall lifetime performance, pigs weaned at 4 weeks of age had a significantly higher DLWG when calculated from birth to slaughter. Whilst there was no effect of weaning age on the number of pigs requiring veterinary treatments from weaning to slaughter, there was a significant increase in the number of 4 week weaned pigs being removed from trial during this period (5.71, 4.36 and 3.88 removals and deaths per 100 pigs for 4, 6 and 8 week weaning P=0.05)

Table 1 The mean physical performance of pigs weaned at 4, 6 and 8 weeks of age (DLWG – Daily liveweight gain, FCR – Feed conversion ratio).

	Weaning Age			sed	P
	4 weeks	6 weeks	8 weeks		
Wean–30kg					
Wean weight (kg)	8.21	12.11	17.39	0.17	***
DLWG (kg)	0.47	0.51	0.59	0.007	***
Feed/pig/d (kg)	0.78	0.88	1.01	0.013	***
FCR	1.67	1.76	1.76	0.023	***
30-90kg					
DLWG (kg)	0.80	0.78	0.77	0.011	*
Feed/pig/d (kg)	2.21	2.15	2.14	0.031	*
FCR	2.84	2.84	2.86	0.048	NS
Lifetime Performance					
Backfat at slaughter (mm P2)	11.15	10.77	10.72	0.073	***
DLWG birth-slaughter (kg)	0.61	0.60	0.60	0.002	**

Conclusion Whilst there were significant benefits to later weaning in terms of piglet performance during the immediate postweaning period, this was not associated with improved clinical health status. When physical performance was considered over the period from birth to slaughter, there were no benefits of later weaning.

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The influence of genetics and environment on indicators of piglet pre-weaning survival

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Introduction Recent estimates of total pre-weaning piglet mortality range between 16-19% (MLC 2006). With environmental modification using the farrowing crate reaching its potential to decrease mortality, as well as raising serious welfare concerns, a different approach to effectively address piglet survival is needed. Genetic breeding programmes implemented in alternative farrowing systems could prove a viable option.

Materials and methods Behavioural and physiological indicators of pre-weaning piglet survival were measured on both gilts (N=65) and their piglets (N=757) in a genetic selection group bred for High (postnatal) Survival (HS) and a Control group (C) farrowing in Indoor loose-housed (I) and Outdoor (O) farrowing systems in a 2x2 design. The gilts were offspring from a previous generation selected for survival traits (Roehe *et al*, 2007) and bred under outdoor conditions. GLMM analysis was used to determine which factors influenced prenatal survival (comparing stillborn piglets with piglets surviving to weaning) and postnatal survival (comparing piglets that were born alive but subsequently died with surviving piglets). GLM analysis was used to compare indicators at a litter level. Potential survival indicators measured included piglet weight and body shape, as measured by ponderal index (PI:birth weight/crown-rump length³), body mass index (BMI:birth weight/crown-rump length²) and abdominal circumference (AC), piglet temperature and behavioural development (e.g. latency to reach the udder, a teat and to suckle), placental traits and gilt behaviours such as posture changes, crushing and aggression.

Results In the O environment total mortality tended to be higher in the C litters than the HS litters (18% vs. 12%, $W_1=3.60$, $P=0.058$), and this tendency was even lower in live-born mortality (C:13% vs. HS:10% $W_1=1.69$ $P=0.193$). In the I environment there were no significant differences in either total mortality (C:12% vs. HS:15% $W_1=0.07$ $P=0.797$) or live-born mortality (C:8% vs. HS :11%, $W_1=0.04$ $P=0.842$). Regardless of environment or genotype, important indicators of prenatal survival were body shape and size (PI: $W_1= 35.50$ $P<0.001$, BMI: $W_1= 37.45$ $P<0.001$ and AC: $W_1=39.97$ $P<0.001$), farrowing birth order ($W_1=10.93$ $P<0.001$) and placental efficiency ($W_1= 6.38$ $P=0.012$). Postnatal survival indicators are shown in Table 1, with birth weight and behavioural development being the most important indicators in multivariate analyses. Indicators were independent of environment, but there were genotype interactions with temperature and time to udder as survival determinants. Gilt behaviour affected piglet postnatal survival: piglets that died had mothers that were more careless with their posture changes (unsupported lying: $W_1=6.37$ $P=0.012$), crushed more ($W_1=5.61$ $P=0.018$) and were more aggressive (Pawing: $W_1=7.06$ $P=0.008$. Rooting: $W_1=4.94$ $P=0.026$ and Biting or Mouthing: $W_1=6.90$ $P=0.009$). When comparing survival indicators at a litter level, regardless of environment, C gilts showed more crushing incidents (both fatal and non-fatal; Figure 1). In the I environment, HS gilts were more aggressive to their offspring and were the only genotype to show savaging ($F_{1,63}=21.83$ $P<0.001$); 40% of the HS-I gilts mouthed or bit piglets.

Table 1 Postnatal survival indicators. GLMM univariate results comparing surviving and dying piglets. Environment (E) and genotype (G) interactions indicated by P-values using GLMM.

Indicators	Wald	P-value	Interactions	
			E	G
Birth weight	15.54	<0.001	0.594	0.161
BMI	6.96	0.008	0.531	0.666
AC	19.50	<0.001	0.437	0.453
2h temperature	5.72	0.017	0.864	0.019
24h temperature	6.34	0.012	0.235	0.027
Time to udder	7.44	0.006	0.548	0.046
Time to teat	10.22	0.001	0.455	0.746
Time to suckle	8.21	0.004	0.511	0.781

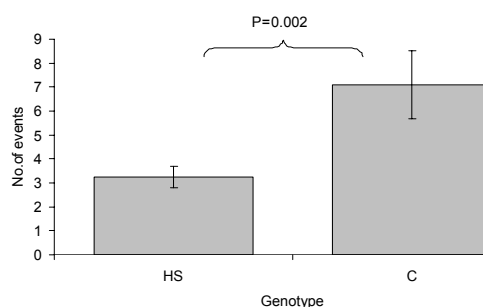


Figure 1 Differences in crushing behaviour between HS and C gilts. P-value using GLM

Conclusions There is potential to breed for high survival in alternative farrowing systems to the crate. The genetic selection programme had the most significant impact in the outdoor environment. The mortality results in the indoor environment illustrates the genotype by environment interactions and the potential to further improve survival. Indicators of prenatal survival were generic across systems, with piglet shape being the most important survival indicator. Postnatal survival indicators included birth weight and behavioural development. In both systems, C gilts showed more crushing behaviour than HS gilts. However, there are potential undesirable side-effects of selecting for HS in the indoor system, with HS gilts showing heightened aggression towards their piglets.

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The relationship between the behaviour of sows and their histories of piglet crushing

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Introduction The crushing of piglets by the sow is the main cause of preweaning mortality and has been reported to be responsible for up to 80 per cent of piglet deaths (Weary *et al.*, 1998). Studies have identified that sows maintain some stable behavioural traits over parities, for example response to a piglet scream test (Grandinson *et al.*, 2003), and sows with a history of crushing piglets are more likely to stay inside a farrowing hut whilst housed outdoors (Johnson *et al.*, 2007). However, there is little information available regarding previous piglet-crushing history and its association with the behaviour of sows housed in farrowing crates. This study compared the behaviour immediately post-farrowing of sows with a history of piglet crushing and those without a single recorded mortality due to crushing.

Materials and methods After studying previous records of piglet crushing, 28 sows were selected for behavioural observation. Fourteen of these had never crushed a piglet before (mean parity with SEM = 3.1 ± 0.27) and were designated NonCrushers (NC). Fourteen of sows had crushed one or more piglets during one of their previous parities (mean parity with SEM = 3.6 ± 0.39 , mean piglets crushed per litter = 0.9 ± 0.29) and were designated Crushers (C). The sows were kept in standard farrowing crates with a piglet creep area and straw provided during the hours of farrowing. The behaviour of each sow was recorded for four hours after the birth of the first piglet with the use of time lapse videos. The frequency and duration of posture changes (lying down on side, shifting while lying on side, lying down ventrally, sitting up, kneeling down and standing up) were recorded. Sow behaviour directed towards the piglets (no reaction, body movement, sits up, stands up, nosing and rooting), particularly after posture changes, was also recorded. Normality of the data was assessed using a Kolmogorov-Smirnov test and data were compared between the two groups using independent sample t-tests in SPSS version 14.

Results Sows with a history of crushing tended to stand up more often (NC vs. C = 5.6 vs. 1.9 ± 2.10 , mean and SEM, respectively, $P = 0.09$). They tended to be less restless whilst lying (NC vs. C = 36.7 vs. 64.6 ± 1.36 , mean and SEM respectively, $P = 0.06$). Otherwise frequency and duration of postures were similar between groups. There were 29 incidences of piglet trapping, 14 by C sows and 15 by NC sows, but only one fatality (by a C sow). C sows were most likely to trap a piglet by sitting on them, whilst NC sows were more likely to trap piglets after lying down ventrally (Figure 1). More piglets were trapped after a shift in lying position (shifting while lying on side) by C sows than NC sows (5 vs. 1 trapped, C vs. NC, respectively) There was virtually no reaction towards piglets during posture changes by any sow from either group, whether they were trapped or not.

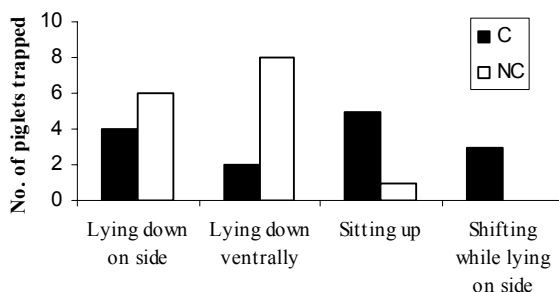


Figure 1 Number of piglets trapped after changes in posture of Crushers (C) and Non-Crushers (NC)

Conclusion Sows with a history of piglet crushing (C) showed different behaviour patterns to those that had never crushed a piglet (NC). Contrary to expectations, the NC sows made more transitions between sitting/lying and standing than the C sows. This has previously been suggested to be the point at which piglets are most at risk from crushing (Weary *et al.*, 1998). However, this may depend on other factors, such as how calm the sow is. In the present study C sows were more restless whilst lying compared with NC sows. In fact, sitting up from a lying position was responsible for 24 percent of piglet trappings, which is higher than recorded by other researchers (Weary *et al.*, 1998). Although the sow's reactions to her piglets has previously been suggested to be an important factor in piglet survival (Grandinson, 2003), there was very little attention paid by C and NC sows to their litter before or after posture changes, regardless of whether a piglet had been trapped. This might suggest that piglet behaviour might play a more important role in influencing the incidence of piglet crushing.

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Influence of management factors during the post weaning period on the performance and behaviour of pigs

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Introduction Producers may wish to house more pigs than normal in a pen in the immediate post weaning period in order to reduce heat and space requirements. The effect of this practice on productivity and behaviour may differ depending on group size used. In addition, housing pigs at reduced space allowances in the immediate post weaning period may lead to a requirement to expand pens or, more realistically, to split groups as the pigs get larger. This study assessed the effects of group size and space allowance during the initial post weaning period, and of expanding pens or splitting groups during the second half of the post weaning period, on productivity and behaviour of pigs.

Materials and methods A total of 1,380 pigs were assigned in a randomised block design to one of six treatments (Table 1). Treatment groups were formed at weaning at 4 weeks of age, at an average weight of 9.4 (SD 1.53) kg. Pigs were housed in combined Stage 1/Stage 2 accommodation with plastic slatted floors. One 4-space feeder supplying dry feed was provided per 20 pigs. This feeder was also provided for groups of 10 pigs, however two of the feeding spaces were blocked off.

Table 1 Description of treatments used (space allowances equate to m² per pig)

Treatment	Stage 1 (4 to 7 weeks)	Stage 2 (7 to 10 weeks)
1	Group size of 20, space allowance of 0.19m ²	Pen expanded to provide space allowance of 0.38m ²
2	Group size of 20, space allowance of 0.38m ²	Same as Stage 1
3	Group size of 40, space allowance of 0.19m ²	Split into two groups of 20, space allowance of 0.38m ²
4	Group size of 40, space allowance of 0.38m ²	Split into two groups of 20, space allowance of 0.38m ²
5	Group size of 40, space allowance of 0.38m ²	Same as Stage 1
6	Group size of 20, space allowance of 0.38m ²	Split into two groups of 10, space allowance 0.38m ²

Individual live weights were recorded at 4, 7 and 10 weeks of age and growth rates calculated. Group feed intake levels were recorded weekly throughout the study, and food conversion ratios calculated. Each group was observed directly for a continuous 2-minute period each hour between 1300 and 1600 hours on 5 days during the first and fourth weeks of the study, and on one day during all other weeks. All occurrences of aggressive behaviours were recorded during these observations. Data were analysed by REML Variance Components Analysis using Genstat 10.2.

Results Performance and behaviour during Stage 1 are listed in Table 2. In general, production performance and aggressive behaviour were higher in groups of 20 rather than 40 pigs (P<0.05).

Table 2 Influence of treatments on performance and behaviour during Stage 1

	Group size (and space allowance)				SED	P
	20 (0.19m ²)	20 (0.38m ²)	40 (0.19m ²)	40 (0.38m ²)		
Feed intake (g/day)	380.2 ^{ab}	417.2 ^b	359.5 ^a	382.7 ^{ab}	19.31	<0.05
Food conversion ratio	1.19	1.24	1.24	1.28	0.050	NS
Growth rate (g/day)	314.8 ^b	321.6 ^b	278.0 ^a	283.8 ^a	12.95	<0.001
7 week weight (kg)	15.8 ^b	15.9 ^b	15.1 ^a	15.2 ^a	0.26	<0.001
Aggression (interactions/pig/obs*)	0.16 ^b	0.17 ^b	0.12 ^a	0.11 ^a	0.019	<0.01

^{a,b} Means in the same row with different superscripts differ significantly, P<0.05. * obs = observation

Performance and behaviour during Stage 2 are listed in Table 3. Food conversion ratio was lower in Treatment 3 than in all other treatments (P<0.05). Aggressive behaviour was lowest in Treatments 4 and 5, and highest in Treatments 2 and 6 (P<0.05).

Table 3 Influence of treatments (1 to 6) on performance and behaviour during Stage 2

	1	2	3	4	5	6	SED	P
Feed intake (g/day)	1123	1125	1068	1102	1115	1109	39.11	NS
Food conversion ratio	1.64 ^b	1.64 ^b	1.39 ^a	1.60 ^b	1.64 ^b	1.64 ^b	0.081	<0.05
Growth rate (g/day)	683.2	685.7	662.6	697.9	681.7	675.9	17.20	NS
10 week weight (kg)	30.0	30.2	29.0	29.9	29.5	30.2	0.56	NS
Aggression (interactions/pig/obs*)	0.17 ^{ab}	0.22 ^b	0.19 ^{ab}	0.16 ^a	0.14 ^a	0.22 ^b	0.028	<0.05

^{a,b} Means in the same row with different superscripts differ significantly, P<0.05. *obs = observation

Conclusions Housing pigs in groups of 40 animals during Stage 1 adversely affected productivity during this stage, but did not have a significant adverse effect on productivity during Stage 2. Aggressive behaviour during Stage 1 was reduced when large groups were used, however treatment effects during Stage 2 were difficult to interpret. Splitting groups of 20 and 40 pigs does not appear to adversely affect productivity, or to promote aggression in weaned pigs.

The effect of group size and floor-space allowance on the efficiency of lysine utilisation by growing pigs

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Introduction Nutritionists formulate feeds based on the genetic potential of the pigs being fed. However, animals may not achieve this potential due to environmental constraints such as: disease challenges, environmental temperatures, feeder space, social stresses and stocking density challenges leading to a reduction in the potential growth rate (Ferguson *et al.*, 2001; Wellock *et al.*, 2006). An experiment was designed in which the potential growth of pigs was reduced to determine whether their nutrient specifications could be reduced concomitantly. Group size and floor-space allowance were used to reduce potential growth rate as it is well established that social stress is one of the most potent factors responsible for the inability of an animal to achieve its inherent growth potential (Ferguson *et al.*, 2001). Here, we assessed the efficiency of lysine utilisation by pigs between 40 and 85 kg live weight, subjected to different degrees of environmental stress.

Materials and methods Two hundred and eighty-eight entire male Large White × Landrace pigs were used. The experiment was divided into two growth periods, i.e. from 40 to 60 kg and from 60 to 85 kg. In each period, pigs were given feed containing one of four dietary lysine concentrations. In Period 1, these were 11.03 (L1); 9.54 (L2); 8.00 (L3) and 6.51 (L4) g digestible lysine/kg feed and in Period 2, 7.82 (T1); 6.71 (T2); 5.55 (T3) and 4.70 (T4) g digestible lysine/kg feed. Pigs fed L1, L2, L3 or L4 in Period 1 were fed T1, T2, T3 and T4 in Period 2, respectively. Six pigs were randomly selected at the start of the trial and slaughtered to determine the initial body composition and two animals per group-housed and four animals per individually-housed treatment (120 pigs in total) at the end of the trial for carcass analysis. Pigs were housed under conditions providing the following group sizes and floor-space allowances: eight pigs per pen at 1.94 m²/pig; four or eight pigs per pen at 1.72 or 0.86 m²/pig respectively; and one pig per pen at 1.72 m²/pig. The individually-housed pigs were divided into three feeding levels, i.e. *ad libitum*, or pair-fed so that feed intakes would match those of *ad libitum*-fed pigs housed in groups of either 4 (restricted-4) or 8 (restricted-8) pigs per pen at 1.72 or 0.86 m²/pig respectively. The efficiency of lysine utilisation is measured by regressing body lysine gain on digestible lysine intake and comparing the amount of lysine required per gram lysine gain with the amount of lysine in the tissue being formed. The appropriate statistical analysis is simple linear regression with groups (Genstat Release 6.1 (2001)); the groups being the various stressors imposed on the pigs. The three stressors used in this trial as possible modifiers of the efficiency of lysine utilisation, were group size, feeding regime and floor-space allowance.

Results For all group sizes, feed intake increased linearly as the dietary lysine content increased. However, this increase was lower ($P < 0.05$) for 8, when compared with 1 and 4 pigs per pen. The linear increase in feed conversion efficiency with dietary lysine content was similar for all group sizes. However, at any dietary lysine concentration, pigs housed in groups of 8 had higher feed conversion efficiencies ($P < 0.05$) than pigs housed individually or in groups of 4. Average daily gain increased linearly as lysine intake increased, this increase being the same for all group sizes. However, pigs housed in groups of 4 and 8 grew slower ($P < 0.01$ and $P < 0.001$, respectively) than those housed individually for any lysine intake. Protein and lysine retentions were unaffected by group size, increasing linearly as lysine intake increased. The efficiency of lysine utilisation (0.45) was not impaired by group size. The pair-fed pigs housed individually (restricted-4 and -8) consumed less feed ($P < 0.001$ and $P < 0.01$, respectively) than the individually-housed pigs fed *ad libitum*, and this was reflected in their average daily gains, which increased linearly as lysine intake increased, but with the restricted-8 growing slower ($P < 0.01$) than the *ad libitum* or restricted-4 pigs. In all three treatments feed conversion efficiency increased linearly with dietary lysine content, although the restricted-4 and -8 had higher efficiencies ($P < 0.001$) than the *ad libitum*-fed pigs at any dietary lysine content. Protein and lysine retentions were unaffected by feeding level and increased significantly with lysine intake. However, at any lysine intake the restricted-8 pigs had a lower efficiency of lysine utilisation ($P < 0.05$) than the *ad libitum* or restricted-4 pigs. The pigs with floor-space allowances of 1.72 m²/pig consumed more ($P < 0.001$) and grew faster ($P < 0.001$) than pigs with floor-space allowances of 0.86 and 1.94 m²/pig at all dietary lysine contents and lysine intakes. Feed conversion efficiency was unaffected by floor-space allowance, and increased linearly with dietary lysine content. Similarly, protein and lysine retentions were unaffected by floor-space allowance and increased linearly as lysine intake increased. The efficiency of lysine utilisation (0.45) remained unaffected by floor-space allowance.

Conclusions These results indicate that when animals are socially stressed, feeding according to the requirement for maximum protein growth produces the best biological performance and carcass composition, with the corollary that, if profitability and biological efficiency is to be maximised, pigs housed in stressful conditions, or those whose future performance is predicted to be below potential because of external stressors, should not be given feed of an inferior quality.

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The effect of drinker design and position on water usage and performance of growing pigs

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Introduction Inadequate water intake is associated with reduced feed intake, poor daily gain, poor feed conversion, scour problems and lower digestibility of feed in pigs (Thacker, 2001). Factors that can reduce water intake include contamination, high mineral content of water, low temperature, low flow rate from drinker, too few drinkers or poor drinker/nipple position (Thacker, 2001). However, there is a lack of information on the effect of drinker design on water intake and performance. Three designs of drinker commonly used are 1) nipple drinkers, 2) bite drinkers and 3) bowl drinkers. Bite drinkers are reported to be less wasteful than nipple drinkers, especially for newly weaned pigs and bowl drinkers are reported to result in less water wastage due to the water being retained in the bowl (Philips and Philips 1999). A reduction in water usage may reduce the volume of slurry produced and is worth further investigation, especially in the light of storage and land spreading restrictions imposed on pig producers by the Nitrates Directive. The aim of the current study was to investigate the effect of drinker design and position on performance and water usage of growing pigs.

Materials and methods A total of 720 $\frac{3}{4}$ Landrace x $\frac{1}{4}$ Large White pigs were weaned at 4 weeks of age and balanced for weight, gender and sire into groups of 20 which were randomly allocated to one of six treatments over six replicates. Two drinkers were placed in each pen of 20 pigs. Pigs were offered water from 4 drinker designs, 2 of which were in different positions. Treatments included 1) Standard Drik-O-Mat bowl drinker – 2 bowls side by side, 2) Standard Drik-O-Mat bowl drinker – 2 bowls placed 2 metres apart 3) Verba nipple drinker – 2 bowls side by side., 4) Verba nipple drinker – 2 bowls placed 2 metres apart, 5) Halfman Bite drinker – 2 drinkers in a forked arrangement, 30 cm apart or 6) Jalmarsen Bite ball Drinkers - 2 drinkers in a forked arrangement, 30 cm apart. All pigs were offered pelleted feed *ad libitum* from dry multi space feeders (one per 10 pigs) (Etra Feeders, Northern Ireland) with a dietary regime as follows: 3kg of Starter 1, 6 kg of Starter 2 and grower diet until 10 weeks of age. Pigs were individually weighed and feed intakes and water use were established at 4, 7 and 10 weeks of age. Daily Feed Intake, Daily Liveweight Gain, Feed Conversion Ratio and Water Usage were determined from weaning to 7 and 10 weeks of age. The results were analyzed by ANOVA using Genstat 8.

Results The design or position of drinker had no effect on growth performance, feed intake or feed conversion efficiency of pigs (Table 1). Flow rates were measured as (mls/min) 250, 600, 700 and 1200 for the Drik-O-Mat bowl, Verba Nipple, Halfman Bite and Jalmarsen Bite Ball drinkers respectively. Compared to the Drik-O-Mat bowl and Verba nipple drinkers, water usage tended to increase from 4 – 10 weeks of age, when the Halfman bite drinker was used and was significantly ($P<0.001$) greater when the Bite Ball drinker was used. Overall, from 4 – 10 weeks of age, when the Drik-O-Mat bowl drinkers were placed apart, water usage was significantly ($P<0.001$) lower than when they were placed side by side.

Table 1 Effect of drinker design and position on pig performance and water usage from 4-10 weeks of age

	Drik-O-Mat		Verba		Halfman Bite	Jalmarsen Bite Ball	Sem	Sig
	Side by side	Apert	Side by side	Apert				
Daily Liveweight Gain (g/d)	495	518	504	495	507	492	8.1	NS
Daily Feed Intake (g/d)	748	752	751	744	756	742	13.1	NS
Feed Conversion Ratio	1.52	1.46	1.49	1.50	1.50	1.51	0.022	NS
Water Usage (l/pig/day)	2.57 ^b	1.84 ^a	2.61 ^{ab}	2.65 ^{ab}	3.27 ^{bc}	3.79 ^c	0.248	***

Within row, means with the same superscript are not significantly different ($P>0.05$)

Conclusion Neither drinker design or position had any effect on pig performance. However, significantly more water was used with the Halfman Bite and Jalmarsen Bite Ball drinkers compared to the Drik-O-Mat Bowl and Verba Nipple drinkers. It is likely that the majority of the extra water used was wasted and hence potentially increased slurry volume, resulting in an additional 17 and 32 tanker loads (6.82 m^3) of slurry produced per year on a 200 sow unit using either the Halfman Bite or Jalmarsen Bite Ball drinkers respectively. This would have major implications on the dry matter of slurry and hence slurry storage requirements on pig farms.

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Outdoor rearing and zinc oxide alter immune status in the newly weaned pig

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Introduction Outdoor reared pigs are said to suffer from less of a growth check post weaning than their indoor reared counterparts (Payne *et al.* 2003) and have more developed gut post-weaning (Carroll *et al.* 2007). Zinc oxide (ZnO) is often used in weaner diets to reduce the incidence of post weaning scours. Previous work by Miller (personal communication) conducted at the University of Leeds identified differences in circulating serum IgG concentrations associated with rearing environment and antimicrobial supplementation post-weaning. Conducted as part of a larger experiment, the aim of this preliminary study was to investigate the effects of ZnO medication of weaner diets upon immune status immediately post-weaning in both indoor and outdoor reared pigs. We hypothesised that piglets reared outside would exhibit a less activated immune response than indoor reared pigs post-weaning and that the presence of ZnO would further reduce immune activation.

Materials and methods Sixteen indoor and sixteen outdoor reared pigs of the same genotype (75%Large White x 25%Landrace) were weaned at 27 ± 0.10 and 28 ± 0.36 (\pm s.e.m) days of age and 9.7 ± 0.36 and $7.8 \text{ kg} \pm 0.26$ liveweight respectively into fully slatted weaner pens balancing for liveweight, sex and litter across treatments. Piglets were offered *ad libitum* access to diets (16.2 MJ DE/kg, 1.6g/kg lysine) medicated with either 0 or 3.1g/kg ZnO. Diets were fed for 13 days post weaning. Pigs were killed at d6 or d13 and lymphocytes were isolated from the mesenteric lymph nodes. Labelled lymphocytes were run through a flow cytometer and the percentage of CD4 and CD8 positive cells recorded. Data were analysed as a 2x2 factorial using the GLM procedures of Minitab 12.2, weaning age and weaning weight were used as covariates.

Results Neither rearing environment nor ZnO inclusion affected the number of CD4 and CD8 positive cells on day 6 post-weaning. There was also no effect of rearing environment or ZnO upon the number of CD4 positive cells on day 13 post-weaning, however the number of CD8 positive cells were significantly ($P < 0.01$) higher for indoor reared pigs by day 13. Medicating the diets with ZnO also resulted in a significant ($P < 0.01$) increase in the number of CD8 positive cells. There was a significant ($P < 0.01$) interaction between rearing environment and ZnO (Figure 1). Outdoor pigs fed diets medicated with ZnO showed a similar immune status to indoor reared pigs fed diets both with and without ZnO.

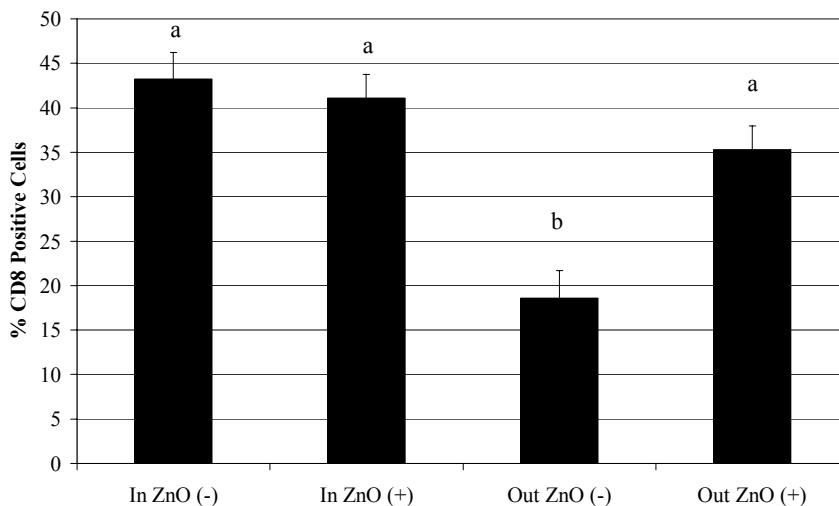


Figure 1 Interaction between rearing environment (In or Out) and ZnO (+ or -) upon CD8 positive lymphocytes on day 13 post weaning.

Conclusions Whilst ZnO feed supplementation and outdoor rearing have both been associated with improved post weaning piglet performance the results of this preliminary trial indicate that this positive effect is achieved in markedly different ways at least with reference to CD8 cell populations. Further investigation of these phenomena is warranted.

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Effect of diet, gender and growth rate on pig meat quality

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Introduction The growth rate of pigs is highly variable both within and between herds (Magowan, 2006) and there is limited research focusing on the effect of variable growth on meat and eating quality. Furthermore, the majority of recent studies investigating the effect of diet on meat quality have focussed on manipulating the fatty acid profile of the meat (Wood *et al.*, 2004). The aim of this study was to assess pig meat quality from gilts and boars offered diets with varying energy and lysine concentrations over a range of average daily gains (ADG).

Materials and methods A total of 120 pigs were commercially housed from 10 weeks of age to slaughter (on average 105kg). Diets were offered to pigs during the finishing period (11 weeks of age to 105kg). The digestible energy and total lysine content of diets 1 and 2 were 13.5MJ/kg, 9.5g/kg and 14.5MJ/kg, 1.1g/kg respectively. Pigs were selected to represent a range of average daily gains (ADG) which were assessed between 10 and 20 weeks of age. On the day before slaughter, pigs were weighed and slap marked. On the day of slaughter pigs were in transport for 1 hour and in lairage for a further hour before they were slaughtered. Pigs were sent to slaughter over 5 time periods and were slaughtered using the normal abattoir procedure. The *Longissimus dorsi* muscle was dissected from the left loin of each pig 24 h after slaughter. Shear Force was measured 7 days post slaughter using a Warner-Bratzler shear device and drip loss over a 48 hour period at 2 days post slaughter on chops of 2.5cm thick. The results were analyzed by Analysis of Variance using Genstat 8.

Results Shear force, drip loss and cooking loss were not significantly affected by either dietary treatment or gender (Table 1). Sarcomere length was significantly longer ($p < 0.05$) in boars than gilts (Table 1). The ultimate pH (pHu) was significantly greater in boars than gilts and the interaction shows that the pHu was higher for gilts on diet 2 but not on diet 1. Average daily gain (10-15 wks) was negatively correlated with cooking loss ($p < 0.05$) with one common line fitting all data, but only explained 3.8% of the variance. Average daily gain (10-15 wks) was also negatively correlated ($p < 0.05$) with WBSF for boars fed diet 2 but positively correlated with WBSF for other sex/diet combinations, however, this relationship only explained 4.5% of the variance. There were no statistically significant correlations between ADG measured at other time intervals (10 to 20 weeks, 15 to 20 weeks, 20 weeks to finish etc) and shear force.

Table 2 Effect of diet and gender on meat quality parameters of the *Longissimus dorsi* muscle in pigs

	pHu	WBSF (Kg/cm ²)	Cooking Loss (%)	Drip (%)	Loss	Sarcomere Length (μ m)
Gender						
Boar	5.49	3.23	30.8	5.8		1.96
Gilt	5.45	3.32	30.6	6.6		1.89
Sed	0.016	0.097	0.57	0.43		0.037
Significance	*	NS	NS	NS		*
Diet						
1	5.46	3.32	30.7	6.2		1.92
2	5.47	3.23	30.7	6.2		1.92
Sed	0.016	0.097	0.57	0.43		0.037
Significance	NS	NS	NS	NS		NS
Interaction						
Gender X diet	*	NS	NS	NS		NS

WBSF Warner Bratzler Shear Force

Conclusion. The dietary treatments used in this study had no effect on any of the meat quality parameters measured. The small differences in pH_u value and sarcomere length between boars and gilts are unlikely to have significant effect in relation to meat quality. The diet x gender interaction for pH_u values may be explained by a small number of boars showing higher pH_u values due to their aggressive nature or attempts at mounting during the pre-slaughter period (Moss, 1978). The variable growth rate of pigs in this study was not reflected in meat quality. However it should be noted that although pigs were slaughtered at an average weight of 105kg, the results may have been confounded by the finish age of pigs which varied by approximately 14 days.

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Utilisation of cassava (*Manihot esculenta*) peel-based diet supplemented with or without Farmazyme[®] 3000 Proenx by growing pigs

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Introduction In view of severe constraints of high cost and sometimes, outright scarcity of maize (the conventional energy source) for pig feeding, the use of some agro-industrial by-products (AIBPs), such as cassava peel is inevitable (Adesehinwa, 2007). However, the fibrous nature of most of the AIBPs has been reported to be a major limitation to their utilisation by pigs (Longe & Fagbenro-Byron, 1990). It has become imperative to incorporate exogenous enzyme(s) into pig diets containing AIBPs, in order to enhance its efficient utilisation by bringing about the breakdown of the limiting non-starch polysaccharides (NSPs) (Ani & Omeje, 2007). It was therefore the aim of this study to determine the effect of the total replacement of maize with cassava peel, supplemented with or without Farmazyme[®] 3000 Proenx on diet quality, as well as, growth performance, efficiency of feed conversion, some carcass traits and haematological indices of the growing pigs.

Materials and methods The utilisation of cassava (*Manihot esculenta*) peel-based diets supplemented with or without Farmazyme[®] 3000 Proenx (a multi-enzyme, containing fungal xylanase, fungal β -glucanase, endo β -glucanase, α -amylase, β -glucanase(pH 7.5, 30[°]C), β -glucanase(pH 5, 30[°]C), hemicellulase, pentosanase and pectinase) was investigated using 36 growing pigs (average initial weight of 22.74 \pm 0.88kg). They were allotted to three dietary treatment groups of (1) 30% Maize-based control diet, (2) 30% Cassava peel-based diet without Farmazyme[®] 3000 Proenx supplementation and, (3) 30% Cassava peel-based diet supplemented with Farmazyme[®] 3000 Proenx. There were three replicates/treatment and 4pigs/replicate (12pigs/treatment) in a complete randomized design. The pigs were allowed *ad libitum* access to the diets and water throughout the 42-day duration of the trial. All the data obtained were subjected to analysis of variance and where statistical significance was observed, the means were compared using the Duncan's Multiple Range (DMR) test. The SAS Computer software package (SAS, 1988) was used for all statistical analysis.

Results The replacement of the 30% maize in the control diet with cassava peel resulted in increased bulk and crude fibre contents of the cassava peel-based diets, hence, lowered energy content and reduction in the dry matter intake of the pigs fed the cassava peel based diet with and without Farmazyme[®] supplementation. The replacement of the maize content of the basal diet with cassava peel resulted in comparable feed:gain but a significant ($P<0.05$) reduction in the cost of feed/kg live weight gain of the growing pigs (Table 1). Farmazyme[®] resulted in enhanced utilisation ($P<0.05$) of the cassava peel-based diet in terms of the daily and overall weight gains. While the RBC and haemoglobin of the pigs were positively influenced by the inclusion of the enzyme, it had no effect on the PCV. The lowered relative kidney weight obtained for pigs on the control was comparable to the diet without enzyme supplementation, while dressing % was not affected by both treatments ($P>0.05$).

Table 1 Performance, cost of feed conversion, relative organ weight, and haematology of growing pigs fed cassava-based diets with or without Farmazyme[®] 3000 Proenx inclusion

Parameters	Cassava peel-based			SEM (\pm)
	Maize-based	with enzyme	without enzyme	
Average Total Weight gain (kg)	16.78 ^a	16.50 ^{ab}	14.39 ^b	0.36
Average Daily Dry Matter Intake (kg)	1.63 ^a	1.42 ^b	1.49 ^b	0.02
Average Daily Weight Gain (kg)	0.40 ^a	0.39 ^{ab}	0.35 ^b	0.03
Feed Conversion (Feed : Gain)	4.51	4.20	4.65	0.13
Av Cost of Feed/Gain (N)(\$1=N130)	115.40 ^a	88.03 ^b	87.26 ^b	0.05
Kidney (%)	0.30 ^b	0.39 ^a	0.35 ^{ab}	0.02
Packed Cell Volume (PCV) %	42.95 ^a	40.95 ^b	39.92 ^b	0.29
Haemoglobin (Hgb) g/dl	14.13 ^a	13.48 ^b	12.37 ^c	0.12
Red Blood Cell (RBC) x10 ⁶ / μ l	7.17 ^a	6.95 ^a	5.97 ^b	0.07

a,b=Means along the same row with different superscripts are significantly ($P<0.05$) different from each other

Conclusion It could be concluded from the results of this study that Farmazyme[®] 3000 Proenx enhanced utilisation of the cassava peel-based diet thereby resulting in performance results comparable to pigs fed the maize-based control diet. Farmazyme[®] 3000 is therefore recommended as a probable feed additive for efficient utilisation of cassava peel-based diets by growing pigs.

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Development of a Terminal Restriction Fragment Length Polymorphism analysis protocol for comparing bacterial populations in the porcine gut

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Introduction Disease in a pig herd can have major economic impacts, hampering agricultural processes and creating barriers to trade. Importantly, an outbreak of disease can also pose a risk to human health. It is currently unknown what effects different rearing regimes might have on the incidences of zoonoses in pigs. Outdoor rearing of pigs has gained popularity recently due to interest in animal welfare and an increase in the marketability of organic food. But it is unknown if outdoor rearing can alter the gut microbiology of pigs, and if pigs reared outdoors are more susceptible to zoonotic infections. A method for analysing bacterial populations present in the pig gut has been developed based on amplification of the 16S ribosomal DNA. This technique, known as Terminal Restriction Fragment Length Polymorphism (T-RFLP) analysis has been used for studying bacterial populations in environments such as soil (Osborn *et al.*, 2000) and faeces (Li *et al.*, 2007). It uses fluorescently labelled forward and reverse primers to generate labelled amplicons, followed by a restriction endonuclease digest of the amplified DNA to give rise to labelled terminal fragments that vary between different species. These terminal fragments are then detected using electrophoretic separation and laser detection, and identified based on the fragment size. This study aims to develop a protocol for using this technique on pure cultures of control organisms.

Materials and methods Pure cultures of *Salmonella enterica* serovar Typhimurium and *Enterococcus faecalis* were obtained from the University of Leeds culture collection, and grown overnight in 10 ml of brain-heart infusion broth. Extraction of the microbial DNA was carried out using the QIAamp DNA stool minikit (Qiagen), and followed by amplification of the 16S ribosomal DNA by PCR using fluorescently labelled forward and reverse primers. The amplicons obtained were purified using the QIAquick gel extraction kit (Qiagen) and subjected to an overnight digest with the restriction enzyme MspI. Digested amplicons were then subjected to capillary electrophoresis using a 3130xl Genetic Analyzer operated using a FA_36_POP-7™ run module and G5 dye set (Applied Biosystems). Data generated from the electrophoresis was analysed using GeneMapper® Software v3.7.

Results Capillary electrophoresis of the *S. Typhimurium* digest detected 2 terminal fragments of the lengths in base pairs (bp) expected from an MspI digest (Table 1, Figure 1). Electrophoresis of the *E. faecalis* digest also detected 2 terminal fragments of the expected length, but also detected a second 5' fragment (Table 2, Figure 2). However, the length of the second fragment suggested it was the product of an incomplete digest at the first restriction site.

Table 1 Terminal fragments obtained from *S. Typhimurium* digest

	Fragment	Length (bp)	Expected length (bp)
S. Typhimurium	5'	493.27	495
	3'	17.95	23

Table 2 Terminal fragments obtained from *E. faecalis* digest

	Fragment	Length (bp)	Expected length (bp)
<i>E. faecalis</i>	5'	563.28	566
	(5')	(617.65)	(625)
	3'	17.59	23

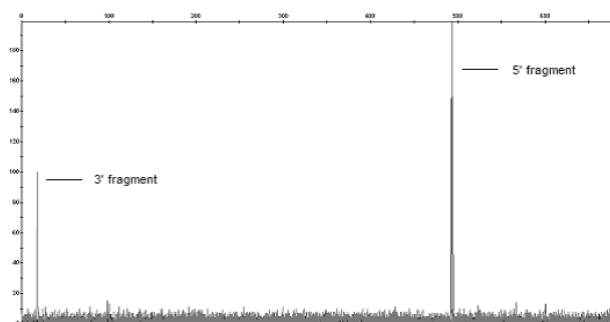


Figure 1 Electropherogram depicting *S. Typhimurium* fragments

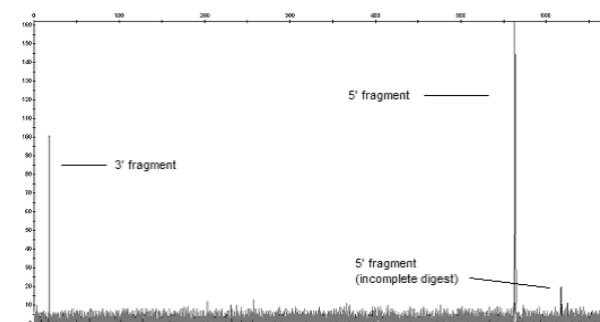


Figure 2 Electropherogram depicting *E. faecalis* fragments

Conclusions A protocol for carrying out T-RFLP analysis has been developed, and shown to work on pure culture control organisms. The restriction enzyme used in this experiment will be changed in order to generate larger terminal fragments, as the current restriction enzyme cuts rather close to the primer binding sequence. This technique is currently being applied to porcine faecal samples to assess the bacterial populations present in the pig gut, and to assess what effects rearing environment might have on these populations.

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Genetic parameters of carcass joint weights estimated from Video Image Analysis measurements

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Introduction The majority of market lamb produced in UK results from crossing terminal sire rams with crossbred ewes. The selection of terminal sire breeds over the past 15 years for improved carcass composition has shown positive benefits on carcass quality of their crossbred progeny (Simm *et al.* 2001). However, faster rates of genetic improvement would be achieved if information from these terminal sire crossbred lambs could be used in the genetic evaluation of terminal sire breeds. Incorporating crossbred information into purebred selection programmes has been modelled and the results show that it is an effective way of improving carcass quality (Jones *et al.* 1999). However, this is currently compromised by limitations on how individual carcass data can be collected for incorporation into breeding programmes. An innovative technology based on Video Image Analysis (VIA) of lamb carcasses is being evaluated for introduction into UK lamb abattoirs. VIA systems can provide an objective, automatic, consistent and accurate way of measuring carcass composition. However, little is known of genetic parameters for VIA measurements of lamb carcasses. Therefore the aim of the present research project was to estimate the genetic parameters of VIA carcass measurements in crossbred lamb population.

Materials and methods A total of 630 crossbred lambs, produced by mating Scottish and Welsh Mule ewes with terminal sire rams (Texel, Charollais and Suffolk), were slaughtered at a fixed point of maturity (estimated fat class 3L; average age 5 months) under commercial conditions at Welsh Country Foods (WCF) abattoir in Gaerwen. Lamb carcasses were presented in a standardized position with the legs spread apart (gambrel) and shoulders banded for VIA scanning of back and side views of carcasses. All lamb carcasses were scanned twice (to estimate repeatability). VIA measurements were identified using the GLM procedures of SAS (SAS Institute Inc., Cary, NC, USA). Fixed effects included: batch (year of birth, sex and farm) and dam age, with age at slaughter as a covariate. Additive genetic effect was fitted as random effect within the model and used to estimate (co)variance components using restricted maximum likelihood (REML) as implemented in ASREML (Gilmour *et al.* 2004).

Results VIA traits analysed show means of 5.58, 3.67, 1.74 and 6.80 with standard deviations of 0.90, 0.65, 0.28 and 0.68 for kilograms of leg, loin breast and shoulder respectively. The effect of age at slaughter on VIA traits was found positive, with values of 4.45, 3.05, 3.29 and 5.02 for weights (gr) of leg, loin, breast and shoulder respectively. Heritabilities for VIA based predictions of kilograms of primal cuts are presented in Table 1. Heritabilities for VIA traits varied from 0.08 to 0.26 with standard errors in the range of 0.06 to 0.10 for shoulder and loin respectively. Genetic correlations involving the leg, loin, breast and shoulder were high >0.79 with standard errors in the range of 0.05 to 0.14. The lowest genetic correlation was estimated between loin and breast (0.79). Phenotypic correlations among traits were ranged from 0.82 to 0.94 (Table 1).

Table 1 Estimates of heritabilities, phenotypic and genetic correlations (standard errors) for VIA based predictions of kilograms of primal cuts[†]

Trait	Leg	Loin	Breast	Shoulder
Leg	0.20 (0.09)	0.86	0.94	0.90
Loin	0.90 (0.07)	0.26 (0.10)	0.82	0.85
Breast	0.93 (0.05)	0.79 (0.12)	0.23 (0.10)	0.86
Shoulder	0.93 (0.09)	0.83 (0.14)	0.91 (0.12)	0.08 (0.06)

[†]Heritabilities on diagonal, phenotypic correlations above diagonal and genetic correlations below diagonal. Standard errors for phenotypic correlations are less than 0.02.

Conclusion VIA based predictions of carcass joints showed moderate heritabilities (except for shoulder), and could be used for selection to improve carcass quality. The estimated genetic correlations were moderate to high for all carcass joints and suggest that genetic improvement in the weight of one joint is in general accompanied by similar changes in the other joints. The use of genetic parameters for VIA traits in the selection for improved carcass quality would be enhanced if they would also be genetically correlated to fatness measurements. Further analysis of measurements of fat and conformation scores, growth traits in addition to the ultrasound carcass traits of muscle depth and fat depth on the same animals will provide additional information about the value of VIA measurements in improving carcass composition by breeding.

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Improving the measurement precision of carcass traits and muscularity in hill breeds using Computer Tomography - impact on maternal performance and lamb survival

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Introduction Breeding indices that combine carcass and maternal performance traits have been tested in practice and result in significant improvements in lamb growth. However, subjective carcass grades for fatness and conformation did not differ as a result of 7 years of selection on the index (Conington *et al.*, 2006). Instrumental grading systems with the potential to objectively and accurately predict carcass composition and muscularity using video image scanning (VIA) may become standard in the UK for lamb carcass grading in the future. The use of computer tomography (CT) scanning of lambs in sheep breeding programmes is likely to better predict the carcass and prime cut VIA-assessed traits and hence lead to accelerated progress in carcass traits. This paper assesses the impact of including CT-derived carcass traits in breeding programmes for hill sheep and the consequences on traits related to maternal traits and lamb survival.

Materials and methods Standard selection index methodology was used to compute the effects of BLUP selection. A typical breeding structure for hill sheep was assumed, using selection intensities of 2.06 and 0.96 for males and females respectively. Genetic parameters among CT-derived lamb carcass and ewe maternal traits for Blackface sheep reported in Lambe *et al.*, (2007) were used together with economic values (EV) taken from Conington *et al.*, (2004). Six index scenarios shown in Table 1 were evaluated. These were 1= 'Terminal sire' index, with two breeding goals only of predicted kg carcass fat weight (CFAT) and predicted kg carcass muscle weight (CMUS) and using *in vivo* measurements of ultrasonic fat and muscle depth; 2= As for scenario 1, but adding CT-measurements of CFAT and CMUS; 3= 'Base index' of 8 breeding goal traits, with the 2 goal and measured traits from scenario 1, plus kg weaning weight (WWT), kg ewe mature size (MS), number of lambs reared to weaning (LWEAN), number of lambs lost from birth to weaning as a trait of the ewe (dead at birth, lambs fostered or removed, LLOST), maternal ability (total weight of lambs weaned, including foster lambs, divided by litter size reared), (MATWWT) and age in years at culling or death (LONGV); 4= As for scenario 3, but adding in CT-measured CFAT and CMUS; 5= As for scenario 3 and including muscularity indices for loin and hindleg (Navajas *et al.*, 2006); 6= As for scenario 3, but substituting LLOST for lamb survival (LAMB SURV) as a (direct) trait of the lamb using the average post-natal survival heritability (h^2) of 0.12 and phenotypic variance (σ^2) of 0.0364 from Sawalha *et al.*, (2006), (*vs* h^2 of 0.033 and σ^2 of 0.2056 for LLOST). The economic value for lamb survival was multiplied by the increase in discounted gene flow coefficient (i.e. was multiplied by 1.6164 *vs* 1.3323, Conington *et al.*, 2004) which increased the EV from -£42.62 to -£51.7. The correlations between lamb survival and the other traits were assumed to be the same as those used for LLOST.

Results Results of the index evaluations are shown in Table 1.

Table 1 Index accuracy and predicted annual responses to selection for each goal trait and total economic response according to each index scenario

	Scenario	1	2	3	4	5	6
Breeding goal (profit) traits							
MS (kg)			0.979	0.962	0.926	0.98	
LWEAN (lamb)			0.017	0.014	0.016	0.017	
LLOST/(lamb)			0.006	0.011	0.009	0.005	
LONGV (days)			0.023	0.021	0.025	0.022	
MWWT (kg)			0.638	0.629	0.641	0.638	
CFAT (kg)	0.002	0.016	0.043	0.027	0.03	0.043	
CMUS (kg)	0.133	0.214	0.133	0.163	0.166	0.134	
WWT (kg)			0.682	0.702	0.7	0.682	
Accuracy of the index	0.48	0.76	0.59	0.62	0.63	0.59	
Economic response (£/yr/100 ewes)	16.9	26.8	106.6	112	113.8	106.8	

trait of the lamb leads to marginal changes in this trait with minimal economic benefit.

Conclusions The use of CT in hill sheep breeding programmes where maternal traits are important components of the breeding programme are unlikely to be cost-effective in the current economic conditions for lamb production in the UK. Due to genetic antagonisms among some of the goal traits, including CT-derived predictions of kg carcass fat, muscle and muscularity may lead to lower responses in maternal performance in extensive environments. Including lamb survival as a direct trait of the lamb *vs* as a trait of the ewe in the index leads to marginal improvements in both lamb survival and economic response.

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If maternal traits are ignored (scenarios 2 *vs* 1) then the use of CT is predicted to lead to a 47% additional response in kg CTMUS or a 56% improvement in economic response. However despite predicted 20% improvements in CT MUS (scenario 4 *vs* 3) including CT in maternal breeding indices leads to only a 5% improvement in economic benefit due to compromises in the responses of other goal traits. Fewer lambs are predicted to be reared /ewe (LWEAN) and more lamb losses (LLOST) are anticipated as a result of including CT-predictions of CTFAT, CTMUS and muscularity (scenario 5 *vs* 3). Including survival as a direct

Crossbred versus purebred ewe genotypes for the hill sheep sector: effects on finishing lamb performance

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Introduction Hill sheep flocks in the UK are dominated by purebred ewe genotypes, with the Scottish Blackface, Welsh Mountain and Swaledale being the most common. However recent changes to the Common Agricultural Policy have led many hill producers to consider keeping crossbred ewes on the hill, with the aim of introducing complementary traits to increase lamb output and improve carcass quality. In 2001 a major on-farm research programme was initiated on 6 hill farms around Northern Ireland to evaluate lifetime performance of a range of crossbred ewe genotypes. Provisional data has already identified that retaining Lleyn X Blackface and Texel X Blackface ewes can improve lamb output at weaning by up to 10% relative to purebred Blackface ewes (Speijers *et al.*, 2007). The aims of the current study were to investigate the effects of switching to crossbred hill ewe genotypes on lamb performance during finishing.

Materials and methods During November 2001 and 2002, 200 purebred Blackface ewes on each of 6 hill farms around Northern Ireland were mated with Blackface (B), Cheviot (CH), Lleyn (LL), Swaledale (SW) and Texel (T) rams. The female progeny from these crosses were retained for breeding, which commenced when ewes were approx. 18 months old. The crossbred ewes were mated to Texel, Lleyn and Dorset rams in single sire mating groups, balanced for ewe genotype, live weight, body condition and age. Over a 2 year period, 588 male and female lambs ($n=296$ year 1, $n=292$ year 2) were moved after weaning to a central location for finishing. Lambs (33 ± 6.8 kg) were permanently housed in groups of six and finished on one of four ad lib concentrate diets comprising grass nuts (T1), standard barley/soya based-concentrate (T2), standard concentrate + rapeseed meal (T3) and standard concentrate + fish oil (T4). Treatments were balanced for ewe genotype, ram genotype, farm of origin and sex. Before finishing commenced, 122 lambs ($n=60$ year 1, $n=62$ year 2) representing all farms and genotypes were slaughtered to enable initial carcass weight to be estimated. Concentrate intake was recorded on a daily basis. Lambs were weighed at weekly intervals until they reached their allocated slaughter weight of 42 or 50 kg for male lambs and 38 or 46 kg for female lambs. Daily live weight gain was determined by linear regression. Cold carcass weight, dressing proportion and grade were recorded at the point of slaughter. Carcass conformation was scored on a 5-point scale using the EUROP classification system (E=5, P=1) while carcass fatness was scored on a 6-point scale using the Livestock and Meat Commission (NI) classification system (1=1, 2=2, 3=3, 4L=4, 4H=4.5 and 5=5). Daily carcass weight gain was determined from the difference between the final carcass weight and estimated initial carcass weight. Data were analysed using regression analysis with fixed effects for ewe genotype and diet and their interaction, and covariates for year, farm of origin, ram genotype, sex and carcass weight. Means were adjusted to a 20 kg carcass weight.

Results Higher concentrate intakes ($P<0.01$) were recorded for lambs born from Texel X ewes relative to those from Swaledale X ewes, although there were no significant differences in feed conversion ratio between any of the ewe genotypes studied. Lambs from Texel X and Cheviot X ewes tended to have higher daily live weight gains compared with those from Swaledale X ewes ($P=0.07$) but there were no significant effects on daily carcass weight gain or killing-out percentage between any of the ewe genotypes. Carcasses of lambs from Blackface and Swaledale X mothers had poorer conformation ($P<0.001$) compared with the other ewe genotypes. Higher carcass fat scores ($P<0.01$) were observed in lambs from Blackface and Swaledale X ewes relative to those from Texel X and Cheviot X ewes.

Table 1 Effects of hill ewe genotype on the performance of their progeny

Ewe genotype	BXB	CHXB	LLXB	SWXB	TXB	s.e.d	Sig
Concentrate dry-matter intake (kg/d)	1.17 ^{ab}	1.15 ^{ab}	1.16 ^{ab}	1.13 ^a	1.17 ^b	0.019	**
Feed Conversion Ratio	6.06	5.66	6.45	6.28	5.85	0.368	NS
Daily live weight gain (g/d)	225	233	212	205	230	11.4	$P=0.07$
Daily carcass weight gain (g/d)	132	132	127	129	143	6.6	NS
Killing-out %	45.5	45.3	46.0	45.1	45.3	0.44	NS
Carcass conformation score	2.87 ^a	3.04 ^b	3.05 ^b	2.82 ^a	3.13 ^b	0.439	***
Carcass fat score	3.66 ^b	3.40 ^a	3.55 ^{ab}	3.62 ^b	3.40 ^a	0.089	**

BXB, Scottish Blackface; SWXB Swaledale X Blackface; CHXB, Cheviot X Blackface; LLXB, Lleyn X Blackface; TXB, Texel X Blackface. Means sharing the same superscript are not statistically significant ($P>0.05$)

Conclusion The results of this trial demonstrate significant potential to improve the carcass quality of lamb sourced from the hill sheep sector by crossing a proportion of Blackface ewes with upland or terminal sires to produce crossbred replacement females. Retaining crossbred ewes with Cheviot and Texel genes improved lamb carcass conformation and reduced the level of carcass fat cover relative to pure Scottish Blackface lambs. Introducing Swaledale genes into the maternal line however had no effect on lamb performance or carcass quality relative to Blackface ewes.

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Bayesian segregation analysis of faecal egg count data simulated from a stochastic genetic epidemiological model of gastrointestinal nematode infections in sheep

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Introduction Resistance to gastro-intestinal (GI) nematode infection in sheep may be partly attributed to host genetic variation. Faecal egg count (*FEC*) of an individual is an important indicator trait that measures the relative resistance of sheep to GI nematode infections. The *FEC* can be measured easily and repeatedly over short time intervals, and its genetic properties and utility as a selection tool are well understood (Bishop and Stear, 2001). However, *FEC* is the result of a complex series of host-parasite interactions that depend on host genetics and immunological mechanisms, epidemiology of the disease, and other non-genetic measures. The objective of the present study was to determine, *in silico*, underlying host immunological mechanisms that could lead to observable major genes for *FEC*. To achieve that, we have extended the stochastic genetic epidemiological model for nematode infection in sheep as described by Bishop and Stear (2001), and used complex segregation analyses to explore the genetic properties of the simulated *FEC* data.

Materials and methods We have modelled the GI parasite *Teladorsagia circumcincta*, which is a predominant nematode of sheep in UK. Briefly, the basic genetic-epidemiological model framework is as follows. The epidemic is triggered by the ingestion of infective third-stage larvae (L_3) from pasture. Within the host system, the L_3 transforms into L_4 and L_5 stages. The model combines all larval stages together and the number of larvae (L) at t^{th} day is given by: $L_t = L_{(t-1)}(1-\mu_L) + L_{it}$, where μ_L is the host-induced larval mortality per day and L_{it} is the number of larvae ingested on the t^{th} day. The larvae finally establish as the adult parasites and the number of mature parasites (M) at t^{th} day is represented by: $M_t = M_{(t-1)}(1-\mu_M) + L_{(t-j)}(1-\mu_L)^{(t-j)}E$, where μ_M is the host-induced mature parasite mortality per day, j is the time taken by larvae to establish as mature parasites and E is the establishment rate of larvae into mature parasites. The parameter fecundity (F) represents the number of eggs laid per female worm per day. Outside the host system (i.e. in pasture), the eggs hatch to free-living first-stage larvae (L_1) that transform into L_2 and L_3 and the whole cycle continues. The parameters (traits) μ_L , μ_M , E and F are assumed to be regulated by host genetics through between-animal variation and *FEC* data are the result of complex interaction between these traits. A population of 1000 sheep was simulated to form the base population and five subsequent generations were generated by random mating. Each generation faced similar environmental conditions and initial natural challenge dose. The model was run for 180 d and *FEC* data from all individuals along with their pedigree information were captured. The simulated *FEC* data were analysed using complex segregation analyses, implemented through a Markov Chain Monte Carlo algorithm using Gibbs sampling methodology (Janss *et al.*, 1995). First, the nematode model was run assuming the infinitesimal model for all traits and subsequently major gene inheritance was assigned to one trait at a time, keeping other traits under the polygenic model.

Results The mean allelic effect (a), allele frequency (p) and additive variance (σ^2_M) of the putative major gene, polygenic additive variance (σ^2_a), error variance (σ^2_e), heritability (h^2) and total heritability including major gene variance (h^2_{total}) and corresponding standard deviations obtained from the posterior distribution are presented in Table 1. The results showed that major genes segregating for larval establishment, larval and adult parasite mortality were not detectable from *FEC* data since the additive effects and additive variances for the respective major genes were not significantly different from zero. On the other hand, the major gene inheritance for fecundity in adult parasite was detected by segregation analysis though the frequency of the favourable allele was slightly higher than 0.5 as assumed under the present model.

Table 1 Marginal posterior mean (MPM) and standard deviation (SD) for different parameters under known major gene

Known major gene	Parameters	a	p	σ^2_M	σ^2_a	σ^2_e	h^2	h^2_{total}
All polygenic	MPM	3.60	0.96	0.07	0.23	0.42	0.36	0.42
	SD	2.19	0.29	0.21	0.09	0.06	0.11	
Establishment (E)	MPM	0.42	0.66	0.02	0.15	0.47	0.24	0.27
	SD	1.55	0.29	0.08	0.07	0.05	0.10	
Fecundity (F)	MPM	0.78	0.63	0.27	0.06	0.41	0.13	0.45
	SD	0.19	0.16	0.11	0.05	0.04	0.09	
Larval mortality (μ_L)	MPM	0.45	0.74	0.04	0.23	0.44	0.34	0.38
	SD	1.43	0.29	0.05	0.09	0.05	0.11	
Mature parasite mortality (μ_M)	MPM	0.32	0.45	0.03	0.21	0.45	0.31	0.35
	SD	0.92	0.29	0.06	0.09	0.05	0.11	

Conclusions The overall results indicated that under the current parameterisation of the genetic-epidemiological model for nematode infections, it was possible to detect the segregation of a major gene for fecundity from *FEC* data.

Acknowledgements The authors thank the Biotechnology and Biological Sciences Research Council for financial support.

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Prediction of prion protein genotypes and study of association with lamb performance traits of Suffolk sheep

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Introduction The relative susceptibility to classical scrapie is mainly determined by polymorphisms at codons 136, 154 and 171 of the prion protein (PrP) gene (Hunter *et al.*, 1996). Five haplotypes, defined by the combination of variants at these three codons are commonly found in sheep; scrapie eradication programmes in several countries are based on changing their frequencies. In Suffolk sheep, one codon shows variation, with the R allele associated with resistance and the Q allele associated with susceptibility. Genotyping costs may be reduced by utilizing information from relatives to infer the genotypes of other relatives. The objectives of this study were to determine the effectiveness of predicting PrP genotypes using information from relatives and to investigate the association of PrP gene with lamb performance traits in Suffolk sheep.

Materials and methods Data were obtained from a Suffolk flock affected with classical scrapie in Scotland. There were 643 animals with known genotypes at codon 171 of the PrP gene. The frequency for the R allele was 47.4%. The genotypes of these animals were utilised to predict the genotypes of 5,173 ungenotyped animals in the pedigree using segregation analysis. A genotypic probability index, as shown in Figure 1 was calculated for each animal based on the amount of information available from other relatives to infer the genotypes (Kerr and Kinghorn, 1996). Association analysis of PrP gene (using animals with both known and inferred genotypes) was carried out using a linear mixed model with random direct and maternal additive genetic effects and maternal permanent and temporary environmental effects was tested and fitted when significant. Several fixed factors were tested for each trait when biologically appropriate and kept in the model when statistically significant ($P < 0.05$). These factors were sex, type of birth and rearing, age of dam, genetic line, breed of dam, year of birth, date of birth, and age at recording (except for birth weight). The expected number of Q alleles carried (from 0 to 2) by each animal was calculated as twice the probability of having QQ genotype plus the probability of having RQ genotype as determined from the genotyping or the segregation analysis and was fitted in the model as a linear and quadratic covariate to test for possible associations with the additive and dominance PrP gene effects, respectively. Estimates of means and standard errors were obtained at expected values of Q allele of 0, 1 and 2 using ASReml programme.

Results The genotypes of 235 animals were inferred with certainty which represents 27% more animals with certain genotypes relative to the known ones (643 animals). About 25% of the 5,173 predicted genotypes were inferred with a genotypic probability index of 50% and over. The genotypes of 262 animals with no information from relatives were inferred with zero accuracy from the Hardy-Weinberg equilibrium frequencies of the population and were not included in the subsequent association analysis. There was no significant association of PrP gene with any of the performance traits analysed (Table 1) as neither the additive nor the dominance effect of PrP gene was significant. There were small differences between the means by expected number of Q alleles carried but they were not statistically significant.

Figure 1 Distribution of the genotypic probability index (accuracy of prediction)

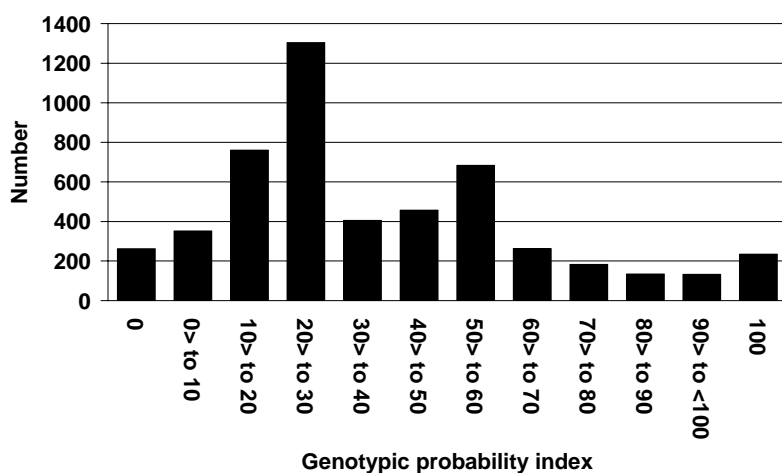


Table 1 Least squares means (s.e.) of different traits by expected number of Q allele as predicted from the linear covariate^a

Trait	Expected number of Q alleles		
	0	1	2
BWt	4.46 (0.09)	4.42 (0.09)	4.37 (0.10)
WWt	22.77 (0.39)	22.57 (0.36)	22.38 (0.41)
Wt150	60.01 (0.91)	59.62 (0.87)	59.24 (0.93)
FD	7.74 (0.23)	7.68 (0.22)	7.62 (0.23)
MD	29.44 (0.30)	29.59 (0.28)	29.74 (0.31)

a: BWt: birth weight, WWt, weaning weight at 56 d, Wt150: 150 d weight, FD and MD: fat and muscle depths at 150 d, respectively.

Conclusions It may be concluded that a considerable proportion of PrP genotypes of Suffolk sheep can be predicted with certainty or with high accuracy from genotypes of relatives. Selection for PrP resistant alleles in Suffolk sheep may not affect performance as the PrP gene may be considered not associated with performance.

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Assessing scrapie risk in dairy sheep flocks

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Introduction Scrapie is a degenerative disorder of the central nervous system of small ruminants. Scrapie in sheep appears to be controlled by genetic factors, with polymorphisms at codons 136, 154 and 171 of the PrP gene locus being the determining parameters (Hunter *et al.*, 2005). Individual PrP genotypes are detected primarily with blood or ear notch tissue samples. Increasingly strict regulations on food safety and public hygiene require new, practical, animal friendly methods for large scale implementation. The first aim of this study was to develop a novel genotyping method using DNA extracted from milk somatic cells from individual animal samples. The second aim was to develop a quick, easy and accurate method for assessing the prevalence of undesirable genotypes within each flock using bulk milk.

Materials and methods Individual milk samples were collected from 850 purebred Chios ewes raised in 19 flocks. Chios is the most productive breed in Greece. Different DNA extraction protocols, one phenol based and three that utilize silica comprising two commercial kits (Nucleospin[®] Blood and Nucleospin[®] Tissue, Macherey-Nagel) and a home made protocol (Borodina *et al.*, 2003) were modified for the genomic DNA extraction from milk somatic cells. Evaluation of these DNA extraction methods was with spectrophotometer measurements, gel electrophoresis and PCR amplification efficiency. Polymorphisms of the PrP gene at codons 136 (Ala or Val), 154 (His or Arg) and 171 (Arg or Gln) were identified by Restriction Fragment Length Polymorphism (RFLP) analysis of Polymerase Chain Reaction (PCR) products using the enzymes *BspHI* and *BspDI*. Two downstream primers reported by Lünken *et al.*, (2004) were used with a new upstream primer PrPov1: GTCAAGGTGGTAGCCACA. At the same time an advanced real-time Ligase Chain Reaction (LCR) protocol was developed for the detection of rare polymorphisms in bulk milk samples. A total of 25 different bulk milk samples were formed by mixing individual milk samples of ewes with known genotypes and the prevalence of undesirable genotypes in each one was assessed. The population was tested for Hardy-Weinberg equilibrium using a chi-square test.

Results Evaluation of the different DNA extraction methods showed that a silica-based commercial kit (NucleoSpin[®] Blood Macherey-Nagel, Germany) with the incorporation of a chloroform extraction step gave the highest quantity and quality of DNA. Results to date, considering 354 ewes that have been individually genotyped, revealed ARQ/ARQ as the most frequent genotype in this population, while ARQ/VRQ, ARR/ARR, ARH/AHQ appeared as the least common genotypes (Table 1). Using these results, population allelic frequencies were estimated as 80.6% for ARQ, 13.6% for AHQ, 4.5% for ARR (the most desirable polymorphism), 1.0% for ARH and 0.3% for VRQ (the least desirable polymorphism). Ensuing statistical analysis showed that this population is in Hardy-Weinberg Equilibrium at the PrP locus ($P > 0.05$). Experiments on bulk milk indicated that the minimum detectable frequency of a polymorphism in the pool was 8% with RFLP and 1% with LCR.

Table 1. PrP genotypes at Chios breed sheep

PrP genotypes	n	Frequencies (%)	Risk group (DEFRA, 2003)
ARQ/ARQ	224	63.3	R3 (genotypes with little resistance to scrapie)
ARQ/AHQ	85	24.0	R3
ARQ/ARR	29	8.2	R2 (genotypes resistant to scrapie)
ARQ/ARH	7	2.0	R3
AHQ/AHQ	5	1.4	R3
ARQ/VRQ	2	0.5	R5 (genotypes highly susceptible to scrapie)
ARR/ARR	1	0.3	R1 (genotypes most resistant to scrapie)
ARR/AHQ	1	0.3	R2
Total	354	100.0	

Conclusions The above results can be used for the development of a genotyping programme for scrapie resistance in Chios sheep flocks. LCR seems to be quicker and more reliable method than RFLP for flock genotyping using bulk milk. LCR was easily applicable for testing high number of samples. Most important, low levels of undesirable polymorphisms (e.g. VRQ) could be detected in a background of excess different polymorphisms. The proposed method can provide a useful test for assessing the scrapie risk in milk and milk products produced by a flock avoiding individual animal genotyping. This can enable the labelling and marketing of potentially “scrapie-free” dairy sheep products.

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Sheep with enhanced resistance to parasites did not cause adaptation in *Trichostrongylus colubriformis* following long-term serial passage

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Introduction Gastrointestinal parasitism is estimated to cost the British sheep industry £83M per annum and these losses could be reduced by breeding sheep with enhanced resistance to parasites (Nieuwhof and Bishop 2005). However, there is concern that parasites could adapt to more resistant hosts through the increased selection pressure placed on parasite populations. The aim of this study is to evaluate the potential of *Trichostrongylus colubriformis* to adapt to host genotype following long-term exposure. We hypothesised that *T. colubriformis* would adapt, as measured by egg output, differentially to hosts selected for either increased (IR) or decreased (DR) parasite resistance following long-term serial passage.

Materials and methods Serial passage used 52 rams (27 IR and 25 DR) and 84 infections (42 IR and 42 DR) through 23 parasite generations. The foundation population (G_0) of *T. colubriformis* was synthetic to ensure ample parasite genetic diversity. Each parasite generation generally had two hosts, with faecal worm egg count (WEC) and faecal culture at 21-25 days post-infection (pi). Following culture, larvae were pooled from each replicate host and used to infect the next generation hosts. On average a host animal received 28,000 larvae; however dose was varied slightly according to age (range 167-623 days). IR and DR animals were randomly chosen from CSIRO *Haemonchus contortus* selection line cohorts (Woolaston and Piper 1996) to be within one standard deviation of selection line mean. Ivermectin (0.4 mg kg⁻¹) was administered two weeks prior to infection and animals were housed throughout passage. Animals were removed due to sickness or zero WEC; also contamination of cultures by other worm species resulted in the DR strain lagging 7 generation behind the IR strain. Parasite strains were assessed using WEC three times in a cohort of animals at pasture following a single dose of ivermectin (0.2 mg kg⁻¹) one week prior to testing. The first test measured WEC in selection line animals (IR, DR and control, n=105) aged 11 months at 28 and 35 days pi following a single dose of 15,000 7th generation DR (G_7 DR) or 15th generation IR (G_{15} IR) larvae. The second strain test measured WEC at 21 and 35 days pi following a dose of 15,000 G_{15} DR or G_{22} IR larvae in 9 month old selection line animals (n=106). The third strain test measured WEC in unselected wether hoggets (n=179) at 28 days pi following infection with 20,000 G_0 , G_{15} DR, G_{15} IR or G_{22} IR larvae. All data were transformed using $\log_{10}(\text{WEC}+100)$ to normalise residuals. Linear models, including fixed effects of animal selection line, parasite strain, maternal handicap, sex and first order interactions, were fitted using ASReml (Gilmour *et al.* 2006). Non-significant terms were removed.

Results Resistant serial hosts showed reduction in WEC compared to the DR hosts (5,300 epg vs. 13,500 epg) (Table 1). No generational changes in egg output were detected in the parasite population and thus the interaction between animal genotype and parasite generation was non-significant ($P>0.05$). For the strain tests, resistant animals had reduced WEC ($P<0.05$) on occasions when animal selection line was tested (Table 3). A significant increased egg output was observed for the older generation in the G_{15} DR/ G_{22} IR test and the G_{15} / G_{22} IR strains compared to the foundation population (G_0).

Table 1 Serial passage host ANOVA F-statistics and solutions [$\log_{10}(\text{WEC}+100)$] (s.e.) for faecal worm egg count

	d	F-statistic	Solution
	f		
Mean			4.135 (0.241)
Animal LINE	1	13.32***	-0.402 (0.009)
Parasite GENERation	1	0.17	-0.004 (0.110)
LINE x GEN	1	0.00	
AGE at infection	1	7.04**	-0.001 (0.0005)

Table 2 Larval strain tests ANOVA F-statistics for faecal worm egg count

	d	G_7 DR/ G_{15} IR	G_{15} DR / G_{22} IR	d	G_0 / G_{15} / G_{22} IR
	f			f	
Animal LINE	2	12.52**	2.31 [#]		
Parasite STRAIN	2	0.49	6.87*	3	7.09***
LINE x STRAIN	4	0.74	2.07		
TIME	1	0.53	6.49**		

[#] $P\leq 0.10$

Table 3 Larval strain test solutions for faecal worm egg count [$\log_{10}(\text{WEC}+100)$] (s.e.)

G_7 DR/ G_{15} IR		G_{15} DR/ G_{22} IR		G_0 / G_{15} / G_{22} IR	
Mean	2.97 (0.13)	Mean	3.52 (0.12)	Mean	3.09 (0.06)
DR-Control	0.09 (0.07)	DR-Control	-0.06 (0.04)	G_{15} DR- G_0	0.35 (0.09)
IR-Control	-0.23 (0.06)	IR-Control	-0.09 (0.04)	G_{15} IR- G_0	0.33 (0.09)
IR-DR	-0.32 (0.07)	IR-DR	-0.03 (0.05)	G_{22} IR- G_0	0.29 (0.09)
G_{15} IR- G_7 DR	0.04 (0.05)	G_{22} IR- G_{15} DR	0.10 (0.04)		
TIME	0.02 (0.26)	TIME	-0.06 (0.02)		

Conclusions There is no evidence that egg output in *T. colubriformis* responded differentially to host genotype. Serial passage intensified selection pressure on *T. colubriformis* above that normally encountered under field conditions and a parasite genotype change was observed, but changes were independent of host genotype. Thus, breeding sheep with enhanced parasite resistance did not increase the risk of parasite adaptation.

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Effects of oil source, feed and pH on biohydrogenation of linoleic acid and production of *trans*-10, *cis*-12 conjugated linoleic acid (CLA) *in vitro*

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Introduction *Trans*-10, *cis*-12 CLA is produced as an intermediary during the biohydrogenation of linoleic acid (C18:2 *n*-6) in the rumen and has been shown to be a potent inhibitor of milk fat synthesis in ruminants. The production of *trans*-10, *cis*-12 CLA in the rumen is affected by dietary concentrate: forage ratio (Kucuk *et al.*, 2001), rumen pH and the amount and source of linoleic acid in the diet. However, the interaction between oil source, carbohydrate source and pH on the production of *trans*-10, *cis*-12 CLA is unclear (Beam *et al.*, 2000). The objectives of the current study were to determine the effects of oil source, carbohydrate source and pH on the biohydrogenation of linoleic acid and production of *trans*-10, *cis*-12 CLA *in vitro*.

Materials and methods Three mature sheep fitted with permanent cannula in the rumen were fed 800g/d concentrate with hay offered *ad-libitum*. After 21 d adaptation, rumen fluid was collected, added to 200 ml Pyrex bottles and incubated for 24 h at 39^o C. To each vessel was added 2500 mg defatted grass nuts (G) or 1250 mg maize starch + 1250 mg grass nuts (M) with either a high (H: initial pH 7.0) or low (L: initial pH 5.9) pH buffer. Each combination of feed and buffer was incubated in triplicate with either 300 mg of sunflower oil (SF), soybean oil (SO), linseed oil (LS), fish oil (F) and fish oil (30 mg) + sunflower oil (270 mg); (FS). The C18:2 *n*-6 contents (mg/vessel) of SF, SO, LS, F and FS were 188, 154, 46, 12 and 170 respectively. After 24 h incubation, vessel fluid pH was measured and contents frozen and freeze dried. The freeze dried contents were ground and analysed for fatty acids using gas chromatograph. Biohydrogenation of linoleic acid was calculated as the amount of C18:2 *n*-6 (mg) added minus the amount of C18:2 *n*-6 (mg) in the residue, expressed as a proportion of that added (mg). Data were analysed as a 5 X 2 X 2 factorial design with main effects of oil, feed and buffer and their interactions using Genstat 8 (VSN Int. LTD., Oxford, UK).

Results The final pH of the vessel fluid was higher ($P < 0.001$) in G than M and higher ($P < 0.001$) in H than L (Table 1). Biohydrogenation of linoleic acid was not affected ($P > 0.05$) by either oil or feed source but was higher with H compared to L ($P < 0.001$); (Figure 1a). Production of *trans*-10, *cis*-12 CLA was not affected ($P > 0.05$) by feed source but was higher ($P = 0.038$) in L compared to H and was highest in SF and lowest in F (Figure 1b). Vessel fluid C18-1, *trans*-10 fatty acid content did not differ ($P > 0.05$) between oil source, but was higher in M compared to G ($P = 0.005$) and L compared to H ($P < 0.001$); (Figure 1c).

Table 1 Vessel fluid pH after 24 h incubation *in vitro*

	Treatment				s.e.d	Significance		
	GH	GL	MH	ML		F	B	F X B
pH	6.50	5.62	6.36	5.18	0.071	<0.001	<0.001	0.005

F, B and F X B = main effects of feed, pH and their interaction.

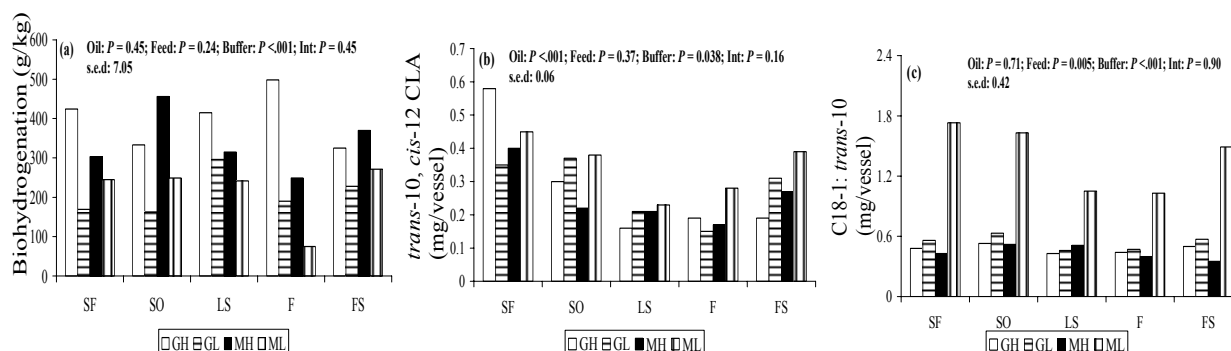


Figure 1 Biohydrogenation (g/kg) of linoleic acid (a), production of *trans*-10, *cis*-12 CLA (b) and C18-1, *trans*-10 fatty acid (c) from different oils incubated with different carbohydrate sources and buffers *in vitro* for 24h.

Conclusions Biohydrogenation of linoleic acid increased with increasing vessel pH. Production of *trans*-10, *cis*-12 CLA was not affected by carbohydrate source but was highest when pH was lowest and with sunflower oil, which had the highest concentration of linoleic acid. Therefore, to manipulate *trans*-10, *cis*-12 CLA production, dietary linoleic acid supply and factors interfering rumen pH are most important.

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Ruminal kinetics of fatty acids and biohydrogenation processes are affected by the amount of extruded linseeds ingested by dairy cows

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Introduction As a result of human nutritional recommendations, experiments have been performed to modify milk fatty acid (FA) composition in order to increase milk content of C18:3 n-3 and to decrease that of both deleterious saturated FA and *trans* FA. The modifications observed when cow's diet is supplemented with a source rich in C18:3 n-3, such as linseeds, are largely dependent on rumen biohydrogenation processes. Indeed, milk *trans* FA and C18:3 n-3 are a consequence of the ruminal biohydrogenation of dietary C18:3 n-3. However, little is known about the effect of increasing amount of extruded linseeds intake by cows on the kinetics of rumen biohydrogenation. This may impact human health because it could affect both the amount of C18:3 n-3 and of *trans* FA available for milk fat production. *Trans* FA profile may be important to consider because their side effect on human health may be due to specific *trans* FA. The aim of this study was thus to assess the effects of increasing intakes of extruded linseeds on kinetics of rumen FA profile, especially *trans* FA.

Materials and methods The experiment was designed as a 4 x 4 Latin square with 4 periods each of 4 weeks, 4 lactating Holstein cows fitted with ruminal cannula and 4 diets. Dietary treatments were made of 50 % natural grassland hay and 50% concentrate without or with 5, 10 or 15 % extruded linseeds (EL, C18:3 n-3 = 54% of total FA) that corresponds to a supplement of 0 (EL0), 2 (EL2), 4 (EL4) or 6 % (EL6) of FA from linseeds (dry matter basis), respectively. The concentrate without or with EL was distributed twice daily (0900, 1630) while hay was offered three times a day (additional time 1330). The forage-concentrate ratio was maintained constant by daily adjustment depending on the refusals of the previous day. Ruminal fluid samples were collected on the last day of each period via the ruminal cannula before (0900) and after meal distribution (1000, 1130, 1400, 1800). FA from feeds and ruminal fluid were prepared as described by Loor *et al.* (2004). FA methyl esters were injected into a gas chromatograph with flame ionisation detector on a CP-SIL 88, 100 m x 0.25 mm i.d. fused silica column, from 70 to 225°C. Results were expressed as g/100g of total FA for each diet. Each kinetics was characterized by its preprandial value (Y_0), its zenith (Y_{max}), by the time at which it occurs (T_{max}) and by the weighted average of g/100g of total FA across time (Y_{mean}). Data were analyzed as a Latin square using the MIXED procedure of SAS (fixed effects: period and diet, random effect: cow). Differences between diets were declared significant at $P < 0.05$.

Results Preprandial value (Y_0) of C18:3 n-3 (g/100g total FA) decreased as the amount of EL intake increased ($P < 0.001$). By contrast, Y_0 of major intermediates of rumen biohydrogenation (IRB) increased as EL increased ($P < 0.01$) except $t11$ C18:1 ($P = 0.19$). After feed intake, C18:3 n-3 increased without effect of EL intake on Y_{max} , T_{max} and Y_{mean} (Table 1) whereas IRB also increased from Y_0 but modifications depended on the FA considered and on EL intake. On one hand, as reflected by Y_{max} and Y_{mean} (Table 1), $t11$ C18:1 and $c9t11$ C18:2 were unaffected by EL intake. On the other hand, some *trans* C18:1 ($t6,7,8$; $t9$; $t13+14$) progressively increased from EL0 to EL4 or rose from EL0 to EL6 ($t11c15$ C18:2 ; $c15$ C18:1). For $t10$ C18:1 only trends were observed. For C18:0, kinetic parameters were not affected by EL intake ($P = NS$).

Table 1 Effect of the amount of extruded linseed intake on individual FA (mean g/100g of total FA) in ruminal fluid (Y_{mean})

	EL 0	EL 2	EL 4	EL 6	S.E.	Diet effect (P)
C18:3 n-3	2.19	3.80	3.03	3.05	0.48	0.22
C18:2 n-6	6.69 ^a	4.6 ^b	3.08 ^c	2.80 ^c	0.63	0.02
<i>c9t11</i> C18:2	0.65	0.75	0.60	0.63	0.16	0.92
<i>t11t13</i> C18:2	0.09 ^a	0.31 ^b	0.33 ^b	0.29 ^b	0.02	<0.001
<i>t11c15</i> C18:2	0.44 ^a	2.34 ^b	4.11 ^c	6.09 ^d	0.44	<0.001
<i>t6,7,8</i> C18:1	0.50 ^a	0.72 ^b	0.98 ^c	1.00 ^c	0.06	<0.001
<i>t9</i> C18:1	0.31 ^a	0.43 ^b	0.54 ^c	0.52 ^c	0.02	<0.001
<i>t10</i> C18:1	0.81	1.14	2.31	4.61	0.92	0.07
<i>t11</i> C18:1	4.78	6.85	9.34	7.57	1.23	0.17
<i>t13+t14</i> C18:1	1.64 ^a	3.36 ^b	4.80 ^c	5.26 ^c	0.34	0.001
<i>t17+c15</i> C18:1	0.11 ^a	0.47 ^{ab}	0.81 ^c	1.22 ^d	0.11	0.002
C18:0	41.28	41.16	39.79	37.60	2.38	0.69

Adjusted mean values within rows with different superscripts significantly differ ($P < 0.05$)

Conclusion These data suggest differential responsiveness of biohydrogenation intermediates when the amount of extruded linseed intake by cows increases. Indeed, among *trans* FA, the relative importance of $t11$ C18:1 is reduced as that of $t11c15$ C18:2, $t13+t14$ C18:1 and $t15+c15$ C18:1 increases. By contrast, increasing extruded linseed intake by cows does not modify C18:3 n-3 escaping from biohydrogenation and C18:0, an index of the final step of biohydrogenation.

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Polyphenol oxidase activity and protein complexing in damaged red clover herbage

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Introduction The level of red clover polyphenol oxidase (PPO) activity can be greatly increased following cell damage. This is probably due to activation of latent PPO by quinone products of phaeolic acid produced by the constitutive active PPO, which is normally at a much lower level than the latent PPO. This “self activation” of PPO following tissue damage could act as an indicator of the level of tissue damage in a conditioned red clover crop. This study investigated a range of tissue damage levels with the PPO activation state and activation with bound-phenol formation in an attempt to correlate degree of cell damage with PPO activity.

Materials and methods Red clover herbage (cv. Milvus) grown in controlled conditions were harvested at approximately 8 weeks regrowth. Leaf tissue (200 g FW) was removed and 50 g sub samples subjected to one of four levels of damage: i) undamaged (UD), ii) lightly damaged (crushed, LD), iii) heavily damaged (blended, HD), iv) frozen in liquid nitrogen and thawed (FT). Each set of samples was left at room temperature for 30 min and then split for analysis of degree of cell damage, PPO activation and production of bound phenols, each analysis was carried out in triplicate. Cell damage was measured using two models: i) loss of electrolytic cellular components measured through conductivity or ii) loss of water-soluble carbohydrate. PPO activity and bound phenols were assayed according to the methods of Robert *et al* (1995) and Winters and Minchin (2005), respectively. Comparisons across levels of cell damage were subject to ANOVA performed with Genstat Release 8.11 (PC/Windows XP; Lawes Agricultural Trust, 2005, Rothamsted, UK).

Results Both cell damage models showed an elevation ($P < 0.05$) in WSC loss and conductivity, with increasing cell damage moving from UD to FT. LD and HD were both greater ($P < 0.05$) than UD and lower ($P < 0.05$) than FT but were not different ($P = 0.876$) from each other in both models. PPO activity and activation for the four levels of tissue damage are shown in Figure 1. Activity and activation is increased with increasing cell damage from UD to FT although there is no significant difference between LD and HD. The relationship between PPO activation and cell damage correlated strongly using both cell damage models: Activation (%) = $0.7425 \times \text{Cell damage (\% conductivity loss)} + 20.454$ ($R^2 = 0.876$) and Activation (%) = $0.7243 \times \text{Cell damage (\% WSC loss)} + 21.933$ ($R^2 = 0.863$). Figure 2 shows a high correlation between PPO activation and bound phenol production with an R^2 of 0.975.

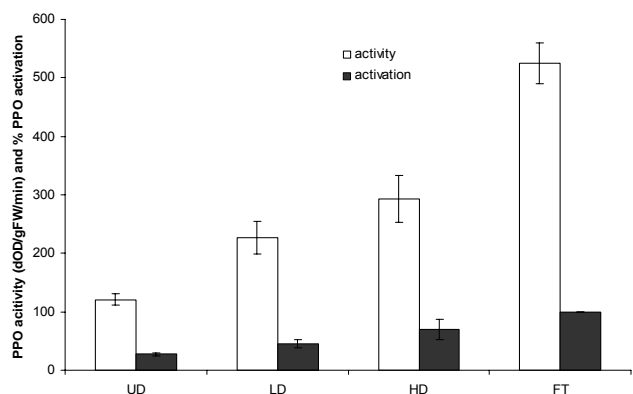


Figure 1 Activity and activation of PPO with increasing cell damage

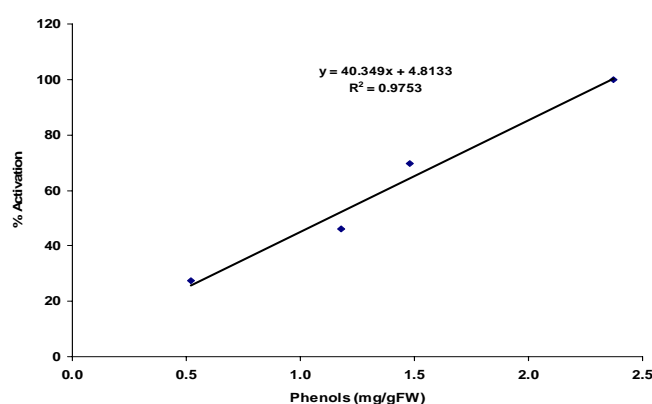


Figure 2 Activation vs. bound phenol production

Conclusions Cell damage was highly correlated with PPO activity and activation and subsequently activation was highly correlated with bound-phenol production. Cell damage is thought to have three mechanisms for increasing PPO activity: i) decompartmentation of intracellular membranes with a subsequent mixing of enzyme and substrate resulting in activation of latent PPO; ii) up-regulating of substrate formation induced through signal compounds, resulting in a substrate induced activation of the latent PPO; iii) up-regulation of the PPO gene increasing enzyme production. These three mechanisms may explain the greatest activation of PPO through freezing and thawing as this technique would increase PPO activity by all three mechanisms whereas as a rapid maceration may prevent an increase in PPO gene expression and thus limit overall activity.

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Rumen microbe adaptation to red clover polyphenol oxidase protein and lipid protection

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Introduction Polyphenol oxidase (PPO) had been shown to reduce both proteolysis and lipolysis in incubated red clover (Lee *et al.* 2004). However it has not been determined whether rumen microbes can adapt to utilise PPO protected protein and lipid. This study investigated whether rumen inoculum from cows offered red clover silage resulted in higher levels of proteolysis and lipolysis in red clover (+PPO) and also in red clover with the PPO1 gene silenced (-PPO) (Sullivan and Hatfield, 2006), than rumen inoculum from cows offered grass silage, due to microbial adaptation.

Materials and methods PPO1 gene silenced (PPO-) and wild type (PPO+) plants grown in controlled conditions were harvested at 5cm above soil level, crushed and cut into 5mm strips and wilted for 1h. Two rumen fistulated cows were offered *ad libitum* either red clover silage (RC) or grass silage (G) for two 2 week periods in a 2 x 2 Latin Square. One litre of hand squeezed rumen inoculum was collected from each cow at the end of each period and transferred back to the laboratory in a temperature regulated flask (39°C). At the end of the first period the cows were given 2 weeks rest before starting the alternative silage in the second period. Red clover (2.5 g) was weighed into 32 tubes, 16 PPO+ and 16 PPO- (for each period) which provided duplicate tubes at each time point (0, 2, 6, and 24 h) for each period so across periods n=4 per time point. Into each tube 7.5ml of anaerobic Van Soest buffer, 0.35 ml reducing agent and 2.5 ml of either RC or G strained rumen inoculum were added before being purged with CO₂ and incubated at 39°C. At each time point the supernatant was sub-sampled for free amino acid analyses. The tubes were then frozen with liquid N₂, freeze-dried and the lipid extracted with 3 x 5ml of chloroform : methanol (2:1; v/v) and dried down under N₂ at 50°C. Membrane lipid and other lipid fractions were determined using thin layer chromatography and then bimethylated before being run on GC. Lipolysis was calculated as the proportional loss of membrane lipid, and rise in free amino acids used as a predictor of proteolysis, with repeated measure analysis of variance used to infer differences.

Results Figure 1 and 2 show lipolysis and rise in free amino acids (proteolysis), respectively of the two red clovers + and - PPO in either RC or G silage fed rumen inoculum. In both rumen inoculums the level of lipolysis and proteolysis were lower (P<0.05) in the PPO+ treatment than the PPO- treatment over time. RC inoculum did not result in a higher level of proteolysis or lipolysis than the G inoculum.

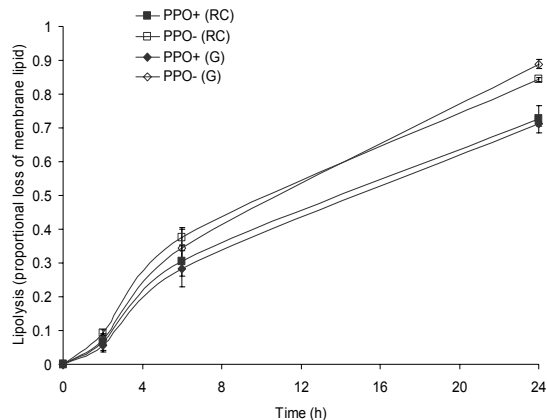


Figure 1 Lipolysis of the 4 treatments PPO + or - with RC or G inoculum

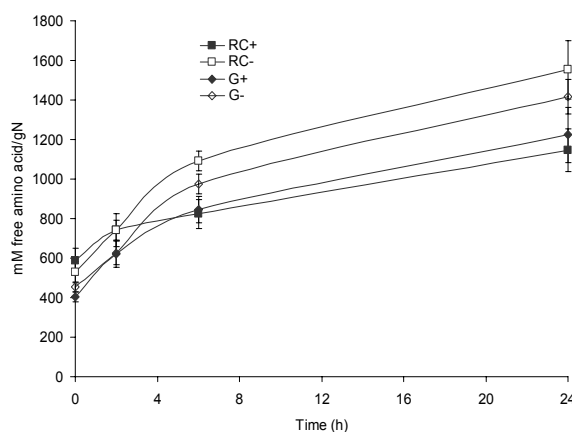


Figure 2 Proteolysis of the four treatments PPO + or - with RC or G inoculum

Conclusions Lipolysis and proteolysis in PPO+ was significantly lower than PPO- in both inoculum regimes with no difference between RC or G inoculum. The results suggest that rumen-micro-organisms grown in an environment containing PPO protected phenol-bound protein do not adapt to increase the utilisation of the protected protein to any great extent.

Acknowledgements This work was funded by an EU framework VI project: PROSAFEBEEF and the Department of the Environment, Food and Rural Affairs.

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Mechanism of polyphenol oxidase action in reducing lipolysis and proteolysis in red clover during batch culture incubation.

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Introduction Lee *et al.* (2007) showed that red clover; with the PPO1 gene silenced (Sullivan and Hatfield 2006), exhibited higher levels of lipolysis than the wild type in the presence of rumen micro-organisms. This questioned the hypothetical mode of action of polyphenol oxidase (PPO) being solely deactivation of the plant enzymes and implied some level of protection of the red clover lipid. It was hypothesised that this may be as a result of protein-phenol complexes, formed by the action of PPO, complexing around lipid micelles and so offering a level of protection. This study investigated whether red clover lipid is protected in the absence of such protein matrixes. At the same time levels of free amino acids were monitored in cultures with the protein matrixes formed to determine whether these offer any form of protection from microbial degradation.

Materials and methods PPO1 gene silenced (PPO-) and wild type (PPO+) plants grown in controlled conditions were harvested at 5cm above soil level, crushed and cut into 5mm strips and wilted for 1h. Half of the PPO- and PPO+ material was frozen with liquid N₂ and stored at -20°C. This was used as the protein complex treatment (PC). The other half of the material was freeze-dried, ground and 1 g weighed out into 12 extraction tubes. The lipid was extracted with 3 × 5ml of chloroform : methanol (2:1; v/v) and the extract dried down under N₂ at 50°C. Once dried 0.19 g Zein, 0.1 g Glucose and 0.33 g Cellulose was added to each tube and these acted as the free lipid treatment (FL). The PC material was defrosted and circa 5 g to account for 1 g DM weighed into 12 extraction tubes for both PPO- and PPO+. This gave 48 tubes in total and four treatments (FL PPO+; FL PPO-; PC PPO+; PC PPO-). Into each tube 7.5ml of anaerobic buffer, 0.35 ml reducing agent and 2.5 ml of strained rumen liquor were added before being purged with CO₂ and incubated at 39°C with a set of three tubes for each treatment allocated to each of the sampling time points of: 0, 2, 6 and 24 h. At these time points for the PC tubes only, the supernatant was sub-sampled for free amino acid analyses. The tubes (PC and FL) were then frozen with liquid N₂, freeze-dried and the lipid extracted as before. Membrane lipid and other fractions separated by thin layer chromatography. Lipid fractions were bimethylated and run on GC. Lipolysis was calculated as the proportional loss of membrane lipid, and rise in free amino acids used as a predictor of proteolysis analysed by a repeated measure ANOVA.

Results Figure 1 shows the extent of lipolysis in the four treatments. In the PC treatment PPO had a significant effect in reducing the extent of lipolysis whereas in the FL treatment there was no effect of PPO. Proteolysis was shown to be significantly reduced by the action of PPO (Figure 2).

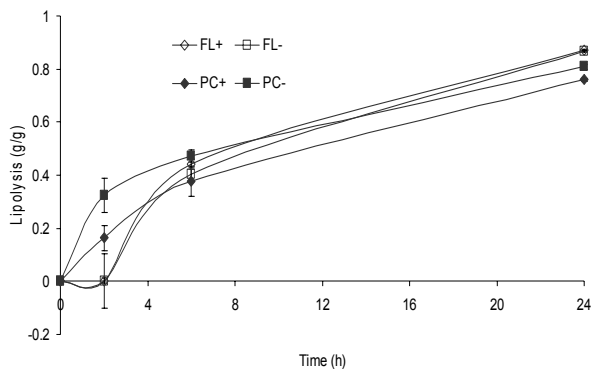


Figure 1 Lipolysis of the 4 treatments PC and LP +/- PPO

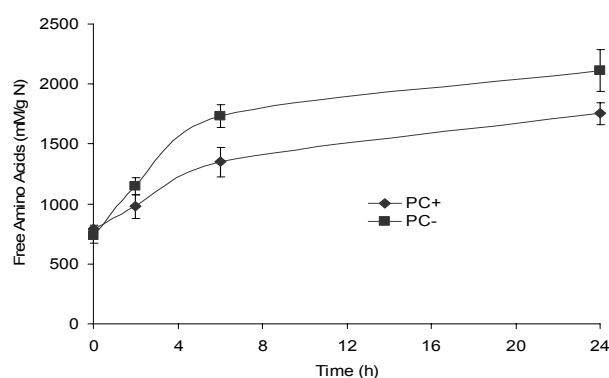


Figure 2 Proteolysis of the PC treatments +/- PPO

Conclusions Red clover PPO significantly reduces proteolysis by the formation of bound-phenol protein complexes. As the protection of forage lipid was lost with the removal of the protein complexes it appears that the reduction in lipolysis by PPO in red clover is due to the protection of lipid micelles within bound-phenol protein complexes.

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Effect of grass species on release of cell components of freshly cut forage during mastication and ingestion

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Introduction As grazing ruminants rely almost entirely on mastication to disrupt plant tissues, a series of processes (mastication, bolus formation and ingestion) will impact on the viability and number of cells that remain intact, and consequently alive, after ingestion (Kingston-Smith and Theodorou, 2000). Preliminary work in our group has shown substantial variation in the degree of cell damage during mastication and ingestion between grass species, resulting in differences in the rate of release of cell contents (protein, sugars and lipids) into the rumen (E.J. Kim, unpublished). These differences may affect nutrient utilisation by ruminal micro-organisms. The aim of this study was to compare the extent of nutrient release from three contrasting grass species following ingestion of the fresh forage by dairy cows.

Materials and methods Three forages, timothy (TIM; cv. Promesse), perennial ryegrass (PRG; cv. AberDart) and tall fescue (TF; cv. Excella) were harvested from field plots. On each of three consecutive days, each forage was offered to two dry, rumen-cannulated, Holstein-Friesian cows following complete rumen emptying. Feed boluses were collected at the cardia. For each cow the sequence in which forages were offered followed a 3x3 Latin square design. Duplicate 50 g sub-samples were transferred into polyester bags (9x23 cm; pore size 40 µm) and rinsed in cold water for 20 minutes using a domestic washing machine. Residues were freeze dried, weighed and then ground prior to determination of total nitrogen (N) and water soluble carbohydrate (WSC). Comparison of the composition of the fresh forage and the washed bolus material allowed evaluation of dry matter (DM) and nutrient solubility following ingestion. The experiment was repeated on three occasions at 6-weekly intervals (Cut 1 - mid May (first cut), Cut 2 - late June (regrowth) and Cut 3 - early August (regrowth)). Effects of forage species were examined by analysis of variance within each cut.

Results Cuts 1 and 3 comprised more leafy material than Cut 2 where all species were at the flowering stage. The chemical composition of the forages is presented in Table 1. Proportional DM disappearance from the boluses was greater for TF ($P < 0.01$), although this difference was not apparent for Cut 2. There were similar patterns for release of N and WSC, with the highest proportion of N (0.52) and WSC (0.76) solubilised from TF.

Table 1 Chemical composition of forages and nutrient solubility following ingestion

		TIM	PRG	TF	SEM [#]	P
Cut 1	N	27.3 (2.92) [§]	25.1 (1.36)	28.3 (1.19)	-	-
	NDF	398.0 (2.58)	365.3 (13.08)	414.8 (5.92)	-	-
	WSC	226.1 (32.21)	269.1 (19.06)	186.6 (13.54)	-	-
	DM solubility (%)	23.6	24.5	40.5	1.96	<0.001
	N solubility (%)	21.7	24.0	40.3	2.54	0.002
	WSC solubility (%)	47.6	46.4	67.8	6.71	0.102
Cut 2	N	19.2 (0.85) [§]	17.1 (1.37)	23.8 (1.00)	-	-
	NDF	629.9 (15.25)	557.6 (17.51)	502.1 (10.08)	-	-
	WSC	79.2 (3.94)	166.6 (5.97)	146.8 (1.72)	-	-
	DM solubility (%)	23.2	33.3	29.6	3.05	0.139
	N solubility (%)	28.9	35.8	35.5	3.76	0.401
	WSC solubility (%)	77.3	65.9	66.8	2.81	0.052
Cut 3	N	33.2 (1.29) [§]	29.2 (0.49)	27.5 (1.71)	-	-
	NDF	491.8 (7.14)	399.1 (8.89)	488.8 (19.86)	-	-
	WSC	79.2 (12.91)	191.8 (2.19)	144.8 (12.16)	-	-
	DM solubility (%)	21.6	25.4	40.7	1.45	<0.001
	N solubility (%)	23.5	31.9	52.3	2.97	0.001
	WSC solubility (%)	47.4	37.6	75.8	3.22	<0.001

[#] 7 degrees of freedom for error; [§] figures in brackets denote standard error of mean (n=3).

Conclusions In general, species differences in N and WSC solubility following ingestion were apparent, but particularly so with leafier material. Resulting differences in actual and relative amounts of soluble nutrients entering the rumen may be reflected in efficiency of capture into microbial protein and overall N utilisation by grazing cattle (see also Kim *et al.*, 2008).

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A comparison of the grassland based systems for mid season prime lamb production from two ewe genotypes

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Introduction In mid season prime lamb production Keady and Hanrahan (2006) concluded that increasing litter size and good grassland management are the main factors affecting efficiency of production. Nolan (1972) reported lamb carcass outputs of 203 and 301 kg/ha from grass-based systems which received 77 kg N/ha and were stocked with 10 and 15 Galway ewes (mean litter size of 1.4 lambs) per hectare, respectively. Subsequently the Belclare breed was developed (Hanrahan 1997) as a genetic resource for increased prolificacy. Recently there has been interest in either reducing or eliminating the winter indoor feeding period by extended grazing. Recent studies at this Centre have shown that extended grazing in mid, late or throughout pregnancy increased lamb birth and weaning weights relative to progeny from ewes which have been housed unshorn (Keady *et al* 2007). The primary objective of this study was to evaluate two contrasting grass-based systems for prime lamb production. The systems adopted compared maintaining the stocking rates and levels of fertilizer nitrogen application similar to that of Nolan (1972) but increasing lamb carcass output through improved genetic capacity and either housing the flock during the winter period or maximising the use of grazed grass by year round grazing. Two ewe genotypes were used to examine if there were any interactions between level of prolificacy and the grassland based system of production adopted.

Materials and methods Two hundred and eighty four ewes of two genotypes [Belclare, Belclare x Cheviot (BxC)] were allocated at random to two systems [grazing and winter feeding (GWF) or year round grazing (YRG)] and remained on the same system for the duration(4 years) of the study, or until they were culled. The YRG system involved extended grazing throughout pregnancy, outdoor lambing, a stocking rate of 10.4 ewes/ha and fertilizer N input of 92 kg /ha. In the GWF system the ewes were housed in mid December and offered grass silage, lambed indoors, turned out at lambing, stocked at 14.1 ewes/ha and involved a fertiliser N input of 92 kg /ha. The same group of terminal sires were used in both systems in each year. The mean lambing dates for the YRG and GWF systems were 30 March and 20 March, respectively. Lambs were weaned at 14 weeks of age. Ewes on both systems were shorn at the same time and received the same treatments for internal and external parasites. The data were analysed using Proc GLM and Proc MIXED of SAS.

Results The effects of system and genotype on ewe and lamb performance are presented in Table 1. There was no evidence for any ewe genotype x system interaction for ewe or progeny performance. Ewes on the YRG system had a higher condition score ($P<0.001$) at lambing. Relative to Belclare, BxC ewes had a higher ($P<0.001$) condition score post mating and at weaning. Lambs from ewes on the YRG system were heavier ($P<0.001$) at birth and weaning and slaughtered at a younger age. System had no effect ($P>0.05$) on lamb carcass weight. Relative to the BxC genotype, Belclare produced larger litters ($P<0.001$) and reared more lambs per ewe mated. Lambs from Belclare ewes were lighter at birth ($P<0.01$) but had similar growth rate and weaning weights to lambs from the BxC ewes. Lamb carcass outputs for the GWF and YFG systems, and Belclare and BxC genotypes were 468, 347, 426 and 390 kg/ha respectively.

Table 1 The effects of system and ewe genotype on ewe and lamb performance

	System (S)			Genotype (G)			Sig	
	GWF	YRG	s.e.	Belclare	BxC	s.e.	S	G
Ewe condition score at:								
post mating	3.58	3.58	0.026	3.50	3.66	0.022	NS	***
lambing	3.19	3.40	0.023	3.27	3.33	0.023	***	(P=0.06)
weaning	3.22	3.25	0.025	3.14	3.32	0.026	NS	***
Litter size	2.17	2.24	0.038	2.34	2.07	0.038	NS	***
Number reared per ewe mated	1.77	1.78	0.042	1.86	1.69	0.043	NS	**
Lamb birth weight (kg)	3.97	4.67	0.055	4.24	4.41	0.049	***	**
Lamb weaning weight (kg)	27.9	30.8	0.25	29.2	29.6	0.26	***	NS
Carcass weight (kg)	18.75	18.75	0.068	18.69	18.82	0.070	NS	NS
Age at slaughter (days)	168	156	1.4	162	163	1.5	***	NS

Conclusions It is concluded that the YRG provides an alternative system of producing mid season prime lamb but requires a reduction in stocking of about 25% relative to the GWF system. Use of genotypes producing litters up to 2.34 lambs/ewe are suitable for both YRG and GWF systems. Lamb carcass output of 492 kg/ha was achieved from the GWF system stocked with Belclare ewes.

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Reduction in content of saturated fatty acids in milk fat by inclusion of whole rapeseed in the diet of dairy cows at pasture

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Introduction Consumption of milk and milk products contributes 14-15% of total dietary fat intake by adults in Great Britain, and provides 23-25% of total saturated fat intake. The relationship between dietary fat intake and incidence of coronary heart disease is well established and current recommendations are that total fat consumption should not exceed 35% of daily food energy intake and that saturated fat intake should not exceed 11% of daily food energy. Studies have shown that different saturated (SFA) fatty acids can have different effects on plasma lipoprotein cholesterol levels and that replacing SFA and *trans* (TFA) fatty acids with cis-unsaturated (USFA) fatty acids has a favourable effect on total and LDL-cholesterol levels (Zock, 2006). The present study aimed to apply dietary modification of the dairy cow diet to produce milk fat with a healthier fatty acid profile to meet human dietary recommendations.

Materials and methods Thirty-two mid-late lactation Hereford x Friesian dairy cows grazing fresh pasture day and night, were allocated to one of four dietary treatments supplying 0, 400, 600 and 800 g rapeseed oil/cow/day on a continuous eight-week feeding trial. The rapeseed oil was presented as whole rapeseed incorporated into a pelleted concentrate which, along with a low oil control concentrate, gave equal total quantities of metabolisable energy and crude protein from concentrate. Animals rotationally grazed a perennial ryegrass sward and were moved to a new allocation daily. Composite am and pm milk samples were collected from individual cows each week of the trial for fatty acid methyl ester analysis by capillary column gas-liquid chromatography (Fearon *et al.*, 2004). Repeated measures of analysis was applied to the fatty acid data set to test for the main effects of diet and week of trial, and their interaction. Contrasts were calculated to test for linear and quadratic effects of increasing the level of rapeseed oil supplementation in the diet.

Results Increasing the level of rapeseed oil in the diet significantly ($P < 0.001$) decreased the proportion of *de novo* synthesised milk fatty acids, C4:0-C14:0. Content of the principle SFA in milk fat, C16:0, and the most hypercholesterolaemic fatty acid, C12:0, were significantly ($P < 0.001$) reduced by rapeseed supplementation, from 328.5 and 32.5 g/kg total fatty acids in control milk to 203.4 and 14.1 g/kg total fatty acids respectively in milk produced from 800g/day rapeseed supplementation. This was accompanied by a significant ($P < 0.001$) increase in the monounsaturated fatty acid, C18:1, from 227.5 g/kg total fatty acids in control milk to 354.6 g/kg total fatty acids in milk from 800 g/day rapeseed supplementation. Overall, total SFA in the milk were reduced from 665.6 g/kg total fatty acids in control milk to 513.7 g/kg total fatty acids in milk from animals on the highest level of rapeseed supplementation. No health problems were observed during the trial and fatty acid changes were established by week 2 and maintained throughout the 8 week period. Linear relationships were established for the main fatty acids; those for C16:0 and C18:1, and total SFA and total USFA (Figure 1), were practically mirror images of each fatty acid/group.

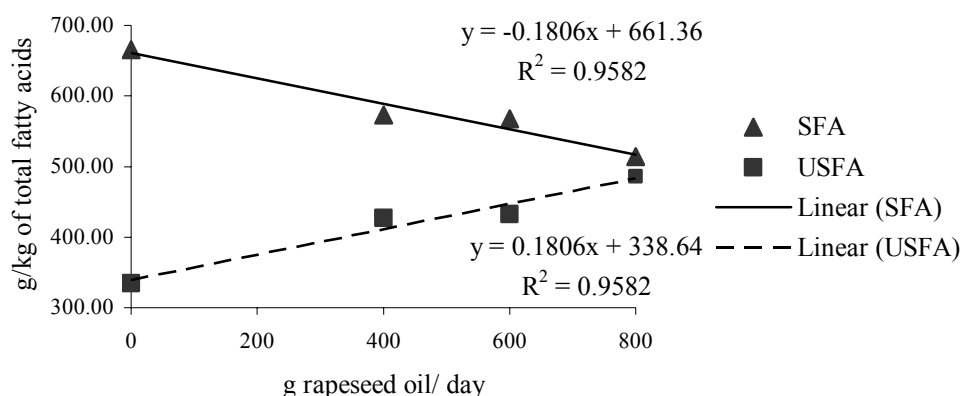


Figure 1 Reduction in SFA and increase in USFA in milk fat with increase in rapeseed supplementation

Conclusions Total SFA content of milk fat can be reduced by over 20%, with a concomitant increase in total USFA of 45% and C18:1 of 35%, by supplementation of the dairy cow diet with whole rapeseed providing 800 g oil/cow/day. This change in fatty acid profile could have important implications for consumer health when the volume of milk and milk products consumed is taken into consideration. Although TFA in the milk fat also increased under the rapeseed dietary regime health professionals consider SFA should be the primary focus of dietary modification because saturated fat consumption is proportionately much larger than that of TFA (Zock, 2006).

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Effect of gamma irradiation on glucosinolate and erucic acid content of canola meal

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Introduction Canola meal (the oil-free residue of low glucosinolate, low erucic acid rapeseed) is a good source of protein for animals and is a particularly rich source of the sulphur containing amino acids, methionine and cystine. Canola meal has some antinutritional factors that they may be responsible for low utilisation of nutrients in the canola meal. These antinutritional factors are glucosinolates, erucic acid, phytic acid and high levels of fibre. Removal of undesirable components is essential to improve the nutritional quality of meals and effectively utilise their full potential as animal feed. Several conventional food processing methods such as germination, soaking, cooking and fermentation are known to reduce antinutritional factors and upgrade the nutritional quality of feeds of plant-origin. However, most of these treatments adversely affect the sensory characteristics of the final product. Food irradiation has been recognised as a reliable and safe method for preservation, improving hygienic quality and improving the nutritional quality of foods (Diehl, 2002). This study was conducted to evaluate the effect of gamma irradiation to reduce glucosinolates and erucic acid content of canola meal.

Materials and methods Samples of canola meal were subjected, at ambient temperature, to gamma irradiation from a ⁶⁰Co source (NORDION, IR-136, Canada) at the Gamma Irradiation Centre, Iranian Nuclear Organization, Tehran, Iran. The applied doses were 10, 20 and 30 kGy, as monitored by radiochromic film. Glucosinolate content of raw and irradiated samples were analysed with a UV-visible spectrophotometer (Varian, CARY 50 Scan, USA) according to Saini and Wratten (1987). Erucic acid was measured with a gas chromatograph (AGILENT, HP6890, USA) using a capillary column (SGE, BPX70, Australia) according to the International Organization for Standardization (ISO 5508: 1990 and ISO 5509: 2000).

Results The data presented in Table 1. shows that gamma irradiation significantly reduced the glucosinolate content of canola meal ($P < 0.01$), where by increasing irradiation dose level, glucosinolate content of canola meal was further reduced. The level of reduction (as percentage of raw canola meal) was 40.29, 70.14 and 89.60 as a function of radiation dose. According to these observations gamma irradiation significantly increased the percentage of erucic acid in total fatty acid content of canola meal ($P < 0.01$). At a dose of 20 kGy, erucic acid had the highest level and then at 30 kGy it was reduced again.

Table 1 Effect of gamma irradiation on glucosinolate and erucic acid content of canola meal

Treatments	Glucosinolate		Erucic acid	
	µmol/g	Reduction* (%)	% in total fatty acid content	Increment* (%)
Canola meal 0 kGy	19.53 ^a	-	0.18 ^c	-
Canola meal 10 kGy	11.66 ^b	40.29	0.26 ^b	44.50
Canola meal 20 kGy	5.83 ^c	70.14	0.29 ^a	58.79
Canola meal 30 kGy	2.03 ^d	89.60	0.27 ^b	48.35
SEM	0.0011		0.00052	

* As % of raw canola meal

Means in the same column with different letter are significantly different ($P < 0.01$).

Conclusion The above observation indicates that gamma irradiation treatment has a significant ($P < 0.01$) effect on the glucosinolate content of canola meal. Gamma irradiation treatment with its radiolytic effects can destroy glucosinolate molecules. Observed results suggested that glucosinolate is very susceptible to gamma irradiation processing, where irradiation at dose 30 kGy decreased its content by 89.60 percent. Reduction of glucosinolate can prevent its adverse effects on thyroid hormones and performance of animals. Although erucic acid is an antinutritional factor, in this study the percent of erucic acid in total fatty acid content of canola meal was increased as a function of irradiation dose level. It seems if the actual concentration of erucic acid in canola meal was measured in different dose levels, we would have better conclusion about gamma irradiation effect on erucic acid. The negative effect of gamma irradiation on fatty acids is main disadvantage of this method for food/feed processing.

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Effect of spice supplementation on rumen ammonia concentration *in vitro*

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Introduction The rumen ammonia level (AL) is the key intermediate in the microbial degradation and synthesis of protein. Measurement of ruminal AL reflects the balance achieved between protein breakdown and synthesis under special dietary circumstances. If the diet is protein deficient or its protein resists degradation, the rumen AL will be low and the rumen microbial growth will be slow. Conversely, if protein degradation is faster than synthesis, rumen AL will accumulate in excess of its optimum level. Excess AL is absorbed into the blood, and converted into urea via liver. Major part of urea is excreted in the urine as a waste causing pollution to the environment. This study examined the effect of various spices when incubated with different forages in rumen fluid (RF) *in vitro* for different times on AL.

Materials and methods Two 4x4x3 factorial experiments in duplicate assessed the effect of four spices (Cinnamon=Ci, Clove=Cl, Cumin=Cu and Turmeric=Tu) at four level (0, 10, 30 and 90 mg/g forage DM) when incubated with three forages (Rice straw=RS, Wheat straw=WS and Hay=H) for two times of 20h and 60h on the AL of RF. RF was obtained from 2 rumen-fistulated sheep just before feeding and transported in insulated flasks under anaerobic conditions to the laboratory. RF was mixed with the pre-warmed buffer at 1:4 ratio to prepare inoculum. Samples of exactly 0.4g dried ground forage were separately weighed into test tubes to which 40 ml of the inoculum were added under CO₂. The tubes were sealed with rubber stoppers containing pressure release valves and incubated at 39°C for the pre-determined times. After each time the tubes were cooled in ice to stop further fermentation. The supernatant was collected after centrifuging the tube at 3000 rpm for 10 minutes and acidified with 1N HCl and kept in a freezer. The acidified samples were analysed for AL by using a colorimeter at 660 nm. The AL data were analyzed by using General Linear Model of Minitab to study the main effects of spice type, spice level and forage and their interaction on the AL at each incubation time and significance declared if P<0.05.

Results The main effects of forages, spices and spice levels were significant for AL at each time (P<0.001). However, due to the significant (P<0.001) interactions between these variables, the mean AL for each treatment combination is shown in Table 1. AL of hay was higher than RS and WS and AL was greatest for Ci and lowest for Cl. AL was greater at 90mg/g forage level than other levels. AL was also increased with the increased time. Rumen AL of donor sheep was 205 mg/l which was within the acceptable AL to maintain rumen function. Tu increased AL at 30mg/g but decreased at 90mg/g forage.

Table 1 *In vitro* rumen AL (mg/l) for different forages when incubated with various spices for different times

Spices	Forages	20h				60h			
		Level of spices mg/g forage				Level of spices mg/g forage			
		0	10	30	90	0	10	30	90
Cinnamon	RS	20.84	34.25	38.83	117.17	30.81	80.44	87.66	360.94
	WS	19.51	41.44	47.97	82.80	48.77	54.08	127.62	364.30
	Hay	30.86	42.06	76.86	115.84	80.00	148.30	134.70	349.60
Clove	RS	20.84	23.59	32.10	45.39	30.81	31.95	46.70	72.83
	WS	19.51	25.40	33.14	39.55	48.77	26.98	33.96	20.28
	Hay	30.86	31.23	38.50	53.13	80.00	87.30	107.30	112.80
Cumin	RS	20.84	21.97	32.98	49.37	30.81	27.29	64.99	121.99
	WS	19.51	27.43	35.58	53.74	48.77	54.35	63.41	185.99
	Hay	30.86	31.78	40.48	66.13	80.00	85.10	115.70	262.00
Turmeric	RS	20.84	20.81	85.28	46.04	30.81	46.35	189.26	110.61
	WS	19.51	34.90	47.01	27.98	48.77	79.72	184.32	93.02
	Hay	30.86	33.52	77.34	59.72	80.00	113.60	149.00	202.70
SEM and Significance		SEM = 50 (P < 0.001)				SEM = 22 (P < 0.001)			

Conclusion It appears that spices can manipulate rumen AL. However, the effect of spices varied with their types and level, incubation time and the forage type. Although Ci contained low CP, the higher AL was perhaps due to two reasons. Firstly, it might have influenced the proteolytic microbes resulting in higher AL. Secondly as it contained less sugars, it might have produced less keto acids which perhaps were unable to utilise ammonia to yield amino acids in the form of microbial protein resulting in increased AL but reduced *in vitro* degradability of cell wall rich forages (Khan and Chaudhry 2008). In Ci, where excessive ammonia is accumulated, supplementing feed containing more soluble sugar may increase amino acid production and thus enhance microbial protein synthesis via increased forage degradation. In general, Ammonia Levels remained in acceptable range with the use of Cumin and Turmeric only.

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Effect of spice supplementation on *in vitro* forage degradability

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Introduction In developing countries, low quality forages, like cereal straws are the main basal feed for ruminants. But these forages are low in energy, protein, vitamin and minerals. Most of the carbohydrates of these forages are bound to compounds like lignin, silica, phenolic, oxalate etc and so the ruminants cannot utilize these forages effectively which affect ruminant health and production. So it is needed to improve the quality of these forages. Herbs and spices which are being safely used for human consumption for a long time may help improve the rumen fermentation and hence the utilisation of these forages if offered to the forage consuming ruminants. This study compared the effects of various levels of four commonly used spices on the *in vitro* dry matter (DM) degradability (IVD) of these selected forages at different times.

Materials and methods Three 4x4x3 factorial experiments, in duplicate assessed the effect of four spices (Cinnamon=Ci, Clove=Cl, Cumin=Cu and Turmeric=Tu) at four level (0, 10, 30 and 90 mg/g forage DM) on the *in vitro* degradability of three forages (Rice straw=RS, Wheat straw=WS and Hay=H) at 3 incubation times of 20, 40 and 60h. Rumen fluid (RF) was obtained from 2 fistulated sheep just before feeding and transported in insulated flasks under anaerobic conditions to the laboratory. The RF was strained through cheese cloth into pre-warmed flasks under CO₂ before mixed with the pre-warmed buffer at 1:4 ratio to prepare inoculum. The flasks were then screw capped and kept at 39°C until used. Samples of about 0.4g dried ground forage were separately weighed into test tubes to which 40 ml of the inoculum were added under CO₂. The tubes were sealed with rubber stoppers containing pressure release valves and incubated at 39°C for the pre-determined times. After each time the tubes were submerged in ice to stop fermentation. The liquids and residues were separated by centrifuging the tubes at 3000 rpm for 10 minutes. Residues were washed with distilled water and dried at 60°C for 48h to determine DM degradability. The IVD data were analyzed by using General Linear Model of Minitab to study the main effects of spice type, spice level and forage and their interactions on the IVD at each incubation time.

Results The main effects of forages, spices and spice levels were significant for IVD at each time ($P < 0.001$). However, due to the significant ($P < 0.001$) interactions between these variables, the mean IVD for each treatment combination is shown in Table 1. IVD of hay was highest and IVD of RS was more than WS with all spices. IVD was highest for Cu and lowest for Ci. IVD was greater at 10mg/g forage level than other spice levels. IVD was also increased with the increased time. At 20h the maximum IVD of H, RS and WS were in the presence of Tu, Cu and Cl respectively. At 40h the maximum IVD of H and RS was found with Cu and maximum IVD of WS was found with Ci. At 60h Tu showed the greatest IVD than other spices. At 20h, Ci, Cl and Tu were more effective at 10mg/g forage level but Cu was more effective at 30 mg/g forage to improve IVD of RS and hay. At 40h Ci, Cl and Tu were more effective at 10mg/g forage and cumin was more effective at 90 mg/g forage. At 60h Tu showed greater IVD than other spices at 10 mg/g forage level.

Table 1 IVD (g/kg) of different forages with different spices at different levels and for 3 incubations (h).

Spices	Forages	20h				40h				60h			
		Level of spices mg/g forage				Level of spices mg/g forage				Level of spices mg/g forage			
		0	10	30	90	0	10	30	90	0	10	30	90
Cinnamon	RS	215	220	193	145	239	336	305	246	467	465	408	334
	WS	229	166	159	138	241	293	256	293	427	317	298	289
	Hay	290	329	235	135	320	424	459	328	567	432	526	360
Clove	RS	215	244	213	245	239	287	280	249	467	402	423	427
	WS	229	203	207	196	241	269	237	271	427	395	413	391
	Hay	290	311	270	287	320	439	413	332	567	511	545	540
Cumin	RS	215	254	276	265	239	338	299	288	467	438	445	443
	WS	229	178	192	199	241	243	261	303	427	415	410	415
	Hay	290	291	326	317	320	360	442	445	567	537	524	513
Turmeric	RS	215	286	257	210	239	291	263	238	467	480	420	462
	WS	229	190	181	219	241	220	223	216	427	446	412	391
	Hay	290	351	293	341	320	322	246	292	567	558	530	560
SEM and Significance		SEM = 50, ($P < 0.001$)				SEM = 27, ($P < 0.001$)				SEM = 22, ($P < 0.001$)			

Conclusions Some spices had positive effects on IVD of different forages. However, the effect of the spices varied with the type and level of spices and the forage type at each incubation time. Cumin and Turmeric contained more soluble sugar which may have enhanced the microbial protein synthesis via increased forage degradation.

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Effect of microbial inoculants on the nutritive value of corn silage for Iranian beef cattle

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Introduction In order to reduce the dependence of the ensiling process on epiphytic lactic acid bacteria (LAB) and on chemical additives, inoculants comprising homofermentative bacteria such as *Lactobacillus plantarum*, *Enterococcus faecium*, and *Pediococcus* species, are often used to control the ensiling fermentation by rapid production of lactic acid and the consequent decrease in pH and improved silage preservation with minimal fermentation losses (Filya, 2004; Muck & Kung, 1997). The objective of this study was to determine the effect of microbial inoculation on the fermentation of corn silage treated with *Lactobacillus plantarum* and *Propionibacterium acidipropionici* silage inoculants and the subsequent effect on nutritive value and performance by lactating beef cattle.

Materials and methods Whole plant corn with 24.2 % DM was harvested and chopped by a conventional forage harvester and was ensiled in two big horizontal silos that had capacity of more than 50 tonnes. Silages were prepared without inoculants (control) or with one of commercially available inoculants (*LALSIL MS01*, *LALLEMAND*, FRANCE). After 30d of ensiling, each silo was opened, sampled and the pH was measured directly on the silage juice, using a pH meter. Six Holstein beef steers (LW=225±17kg) were allotted to 2×2 repeated Latin Square design at two 21d periods (adaptation, 14d, and sample collection, 7d) for evaluation of the effect of microbial inoculation on digestibility of corn silage nutrients. Diets had a similar composition and contained (%) 94.5, 5.0, 0.2, 0.2 and 0.1, silage, dicalcium phosphate, mineral/vitamin supplements, and salt, respectively. Total faeces were collected from all cows for 7d of each period (days 14 to 21). Samples of silage, rations, and faeces were dried at 55°C for 48h in a forced-air oven for DM determination, ground through a Wiley mill (1mm screen pore size), and analysed for DM, crude protein (CP), ether extract (EE), ash and neutral detergent fibre (NDF). Non-fibre carbohydrate was calculated as: 100- (%CP+ %NDF+ %Ash+ %EE). The concentration of NH₃-N was measured with Kjeltac Auto Analyzer.

Results The results of experiment are presented in Tables 1 and 2. Inoculation of silage significantly reduced pH of silage and concentrations of ammonia-N of corn silages compared with the control silage. The NDF content of inoculated silage was significantly lower than control silage which is a result of more fermentation of NDF content of silage in inoculated silage. In addition, the lower NDF content of inoculated silage is result of partial acid hydrolysis of hemicelluloses (Filya, 2004). Accompanying the increased DMI by steers consuming inoculated silage were significant increases in NDF, CP, ether extract and ash digestibility compared with steers fed control silage. Nutrient digestion was different in steers fed untreated or treated corn silage. Inoculated silage had significantly higher digestibility coefficients for all nutrients.

Table 1 The pH and chemical composition of silages

Item	Silage	
	Control	inoculated
pH of corn before ensiling	6.23	6.23
NH ₃ -N concentration (mg/dL)	8.96 ^a	6.25 ^b
pH of silage	4.22 ^a	3.93 ^b
Dry matter (%)	24.55	24.15
Crude protein (%)	8.89	8.82
Neutral detergent fibre (%)	62.22 ^a	58.32 ^b
Non-fibre carbohydrate (%)	19.43 ^b	23.7 ^a
Ether extract (%)	3.20	3.11
Ash (%)	5.98	6.33

Means within a row with different subscripts differ (P<0.05).

Conclusion Addition of microbial inoculants to corn silage improved the nutritive value of corn silage for beef cattle. Inoculation with *Lactobacillus plantarum* and *Propionibacterium acidipropionici* silage inoculant tended to decrease pH and increase DMI and nutrient digestibility.

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Table 2 Effect of microbial inoculants on intake and nutrient digestibility of corn silage-based diets fed to beef steers

Item	Silage	
	Control	Inoculated
Intake(kg/d)		
Dry matter	8.82 ^b	11.04 ^a
Crude protein	1.1 ^b	1.4 ^a
Neutral detergent fibre	5.63 ^b	5.81 ^a
Non-fibre carbohydrate	2.56 ^b	3.20 ^a
Ether extract	0.55 ^b	0.65 ^a
Ash	0.26 ^b	0.33 ^a
Digestibility (%)		
Dry matter	75.03 ^b	79.91 ^a
Crude protein	72.30 ^b	76.35 ^a
Neutral detergent fibre	62.50 ^b	70.56 ^a
Non-fibre carbohydrate	85.66 ^b	89.68 ^a
Ether extract	66.71 ^b	83.26 ^a
Ash	48.08 ^b	65.37 ^a

Means in a row with different subscripts differ (P<0.05).

Effects of different additives on chemical composition of whole crop canola silage

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Introduction Silage making is one of the main conservation methods that is used in forage preservation (Muck, 1993b and Cassida, *et al.* 1994). Application of silage additives is the most important factor in improving silage quality (Muck, 1993a). Canola is one of the crops that produce throughout the world. Since, canola could not reach to stage of blossom in some climates such as cold climate, so farmers should mow and making silage for animal nutrition. The objective of present study was to determine the effects of different additives on chemical composition of whole crop canola silage.

Materials and methods In order to determine the effects of molasses, beet pulp and orange pulp on chemical composition of canola silage, an experiment in a complete-randomized design with three replications was conducted in farm of Ferdowsi University of Mashhad. Treatments were, 1-control 2-10% molasses(on DM basis of canola forage) 3-10% beet pulp(on DM basis of canola forage) 4-10% orange pulp (on DM basis of canola forage). Canola(hyula 308), were seeded in October and mow in June. Forages were chopped in size of 1-2cm, then molasses, beet pulp and orange pulp were added in 10%(on DM basis) and silage were preserved in nylon bags for 60 days. After this period, 4 samples were taken and chemical composition of these were measured. Data were analysed using General Linear Models procedure of SAS V6.12 for ANOVA to evaluate differences among experimental groups, means compared with Duncan test.

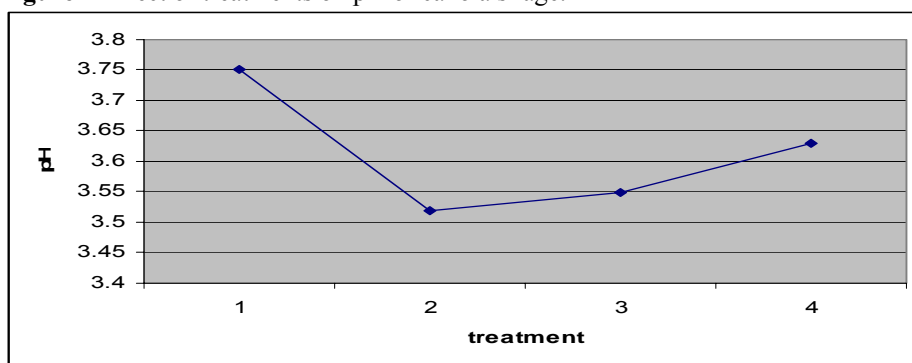
Results DM content of silage with molasses was increased significantly ($P<0.05$), but molasses decreased pH, NDF and ADF of canola silage ($P<0.05$). Treatments didn't have significant effects on CP of canola silage(table1). All of the silages have green to yellow colour and suitable smell, especially in silages with additives.

Table 1 Effects of treatments on chemical composition of canola silage.

Treatment	Control	10% Molasses	10% Beet Pulp	10% Orange Pulp	SEM
pH	3.75 ^a	3.52 ^c	3.55 ^c	3.63 ^b	0.03
DM	22.02 ^d	29.9 ^a	26.07 ^b	24.06 ^c	0.11
NDF	56 ^a	40.33 ^d	50.66 ^b	46.66 ^c	0.68
ADF	42 ^a	31.66 ^b	40.33 ^a	41 ^a	0.48
CP	10.28	10.8	9.65	9.92	0.26
fat	1.3 ^{ab}	1 ^b	2 ^{ab}	2.3 ^a	0.22

^{a, b, c, d} Means in the same row with different letters are different ($P<0.05$).

Figure 1 Effect of treatments on pH of canola silage.



Conclusions Molasses increased pH and decreased DM of canola silage, so It seems that applying soluble carbohydrate in canola silage could be an acceptable replacement with corn silage.

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***In vitro* methods for fibre degradation show how feed enzymes can improve the nutritional value of coproducts from bioethanol production in rations for monogastric animals**

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Introduction Distiller's dried grains with solubles (DDGS) is a coproduct of bioethanol production from cereal grains. Due to the selective removal of starch during the production process, the protein and fibre in DDGS is approximately three times more concentrated compared with the starting material. In Europe, wheat is the main cereal used in bioethanol production and DDGS derived from this process has been traditionally fed to ruminants. Modern bioethanol plants produce DDGS with high protein quality and DDGS is therefore increasingly used in monogastric rations. However, the high fibre content remains a challenge to animal performance. An increased intestinal viscosity is the primary concern when animals are raised on diets with high levels of soluble fibres. Feed enzymes are widely used to neutralise this antinutritional effect. The major part of the dietary fibre in wheat and wheat DDGS consists of insoluble fibre, which does not cause viscosity problems, but traps valuable nutrients in the feed matrix. At least a partial degradation of this fibre is required to release the nutrients in the gastrointestinal tract. Fibre fermentation in the hindgut of monogastric animals may represent a significant energy salvage due to the production of volatile fatty acids and contribute to the maintenance of the intestinal health status. This paper presents an *in vitro* comparison of the functionality of fibre from both whole wheat and wheat DDGS in monogastric nutrition, and shows how feed enzymes may improve the nutritional quality of DDGS.

Materials and methods Wheat of normal viscosity was obtained from a continental feed mill, high viscosity wheat from Great-Britain, and wheat DDGS (Protigrain™) from a German bioethanol plant (Cropenergies). Kemzyme® plus Dry (Kemin Europa; EU registration E1620) was used as a feed enzyme and supplied xylanase, beta-glucanase and cellulase as fibre-degrading activities. A viscosity reduction assay was performed by extracting wheat or wheat DDGS in phosphate buffer pH 6.0, incubating the extract for 1 hour at 40°C, and measuring the viscosity. Dietary fibre was analysed according to AOAC method 991.43 (Lee *et al.* 1992), but an additional preincubation period of 1 hour in acetate buffer pH 4.5 was introduced in order to examine the action of fibre-degrading enzymes. Fibre fermentation was simulated by a digestion of the substrate with pepsin and pancreatin at pH 3 and pH 6, respectively, and fermenting the insoluble residue after digestion in medium as described by Theodorou *et al.* (1987). The fermented broth was assayed for volatile fatty acids and ammonia.

Results The extract viscosity of wheat DDGS was only slightly higher than continental wheat varieties, and it was significantly lower than the typical high viscosities observed for British wheat cultivars (Table 1). The conditions during bioethanol production may have reduced the soluble fibre fraction that is responsible for the viscosity effect. Feed enzymes reduced the extract viscosity of wheat DDGS further to a value similar to enzyme-treated continental wheat. The dietary fibre level in wheat DDGS (333 g/kg) was as expected much higher than the level in whole wheat (139 g/kg). Feed enzymes broke down a significant fraction of the dietary fibre both in whole wheat and wheat DDGS. The levels of unbranched volatile fatty acid after fermentation were related to fibre fermentation, whereas the levels of branched volatile fatty acids and ammonia were related to the fermentation of undigested protein. Higher levels of acetic, propionic and butyric acid produced from DDGS treated with feed enzymes indicated a higher fibre fermentation rate. The increased fibre fermentation coincided with a lower protein fermentation rate, as shown by the lower levels of iso-butyric, iso-valeric acid and ammonia.

Table 1 Effects of feed enzymes on viscosity, dietary fibre and fermentation characteristics of DDGS

		Control	Control treated with enzymes	Difference
Extract viscosity	Normal viscosity wheat (cP)	1.46	1.33	-0.13
	High viscosity wheat (cP)	1.79	1.56	-0.23
	Wheat DDGS (cP)	1.56	1.30	-0.26
Total dietary fibre content	Wheat (g/kg)	139	108	-31
	Wheat DDGS (g/kg)	333	286	-47
Volatile fatty acid and ammonia production from wheat DDGS in gut fermentation model	Acetic (g/kg)	9.19	13.0	3.79
	Propionic (g/kg)	0.59	0.8	0.16
	Butyric (g/kg)	0.75	1.0	0.28
	Iso-butyric (g/kg)	0.91	0.3	-0.57
	Iso-valeric (g/kg)	1.68	0.8	-0.90
	Ammonia (g/kg)	5.17	3.3	-1.88

Conclusions *In vitro* data showed that feed enzymes have the same potential in diets containing wheat DDGS as in traditional wheat-based diets for a reduction of the intestinal viscosity and for the release of nutrients encapsulated in the insoluble fibre matrix of the feed. Feed enzymes may also beneficially modulate the balance between fibre and protein fermentation in the hindgut of monogastric animals that a fed with important amounts of DDGS.

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The effect of non fibre carbohydrate on *in vitro* first order dry matter disappearance model of various ruminant feeds

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Introduction Starch is digested rapidly in the rumen, but more slowly than sugar sources. The effective use of high-sugar products in supplementation programs requires knowledge of their effects on forage use and of how they compare with other common supplemental carbohydrate sources such as starch (Sniffen and Robinson, 1987). The objective of this study was to determine the effect of supplementing sucrose or starch on *in vitro* first order dry matter disappearance model of various ruminant feeds (lucerne, wheat bran and sugar beet pulp).

Materials and methods Experimental feed samples were lucerne, wheat bran, and sugar beet pulp. Samples were ground using a Willey-mill to pass 0.75 mm screen, and dried at 80 °C for 48 h. Non-supplemented or non fibre carbohydrate (starch and/or sucrose) supplemented samples were incubated in a medium prepared as described by Arroquy *et al.* (2005). The supplementation was carried out as 70 mg/g DM of feed sample as starch or sucrose or Starch+ sucrose as 1:1. Forty-five ml of medium (0.40 cell-free rumen fluid and 0.60 mineral mixtures) were distributed into 100 ml bottle containing 1 g of each feed sample. Then, each bottle was inoculated with 5 ml of cloth-cheese strained rumen fluid and finely bubbled with CO₂. Previous to the inoculation, the rumen fluid was incubated for 1 h in an incubation chamber at 39 °C. Rumen fluid was obtained from 4 Holstein steers fed maize silage, lucerne hay, wheat straw, barley grain and soybean meal (3.4, 2.4, 0.8, 1.6 and 0.8 kg/d DM, respectively). Bottles were incubated for 24, 48, and 96 h at 39 °C (4 bottles per each treatment and 2 bottles as blank per incubation). Then, bottle content was filtered through a 22 µm filter paper. Unfiltered dry matter was measured using air forced oven at 80 °C, 48 h. Data were analysed using GLM procedure of SAS and applied to a non-linear first order model to estimate the digestion kinetic parameters. The model was $D_{(t)} = D_{(i)} \exp(-k \cdot \text{time}) + I$; where $D_{(t)}$ is potentially digestible fraction of DM, $D_{(i)}$ is potentially digestible residues, k is fractional rate constant of digestion (h^{-1}) and I is indigestible fraction.

Results First order fractional rate constant of digestion (h^{-1}) and indigestible fraction of none or NFC supplemented of the feeds are shown in Table 1. Indigestible fraction of lucerne hay was significantly increased when it was supplemented by NFC ($p < 0.05$). The lowest constant rate of digestion was observed when a mixture of starch and sucrose (1:1) was added to wheat bran ($p < 0.05$). Constant rate of digestion of sugar beet pulp was also significantly influenced by supplemental NFC ($p < 0.05$). The indigestible fraction of all NFC supplemented sugar beet pulp was lower than non supplemented samples.

Table 1 Effect of non fibre carbohydrate sources on *in vitro* first order dry matter fractional rate constant of digestion (h^{-1}) and indigestible fraction of various ruminant feeds (mean \pm s.e.m.).

* Sucrose or starch was added as 70 mg/g DM; ** 35 mg/g DM of sucrose and 35 mg/g DM of starch was added.

^{a, b} Within each feed, difference between means with different letters is significant ($P < 0.05$).

Feeds	Fractional rate constant of digestion (h^{-1}) [‡]		Indigestible fraction [‡]		R ²
	mean	s.e.m.	mean	s.e.m.	
Luerne	0.109 ^a	0.019	0.529 ^a	0.012	0.977
Luerne+ sucrose *	0.079 ^a		0.570 ^b		0.978
Luerne+ starch *	0.092 ^a		0.571 ^b		0.962
Luerne+ sucrose+ starch **	0.079 ^a		0.570 ^b		0.978
Wheat bran	0.118 ^a	0.009	0.339 ^a	0.004	0.997
Wheat bran+ sucrose	0.150 ^b		0.349 ^b		0.999
Wheat bran+ starch	0.113 ^a		0.338 ^a		0.998
Wheat bran+ sucrose+ starch	0.092 ^c		0.345 ^{ab}		0.997
Sugar beet pulp	0.096 ^a	0.004	0.368 ^a	0.006	0.998
Sugar beet pulp+ sucrose	0.079 ^b		0.335 ^b		0.997
Sugar beet pulp+ starch	0.072 ^b		0.339 ^b		0.997
Sugar beet pulp+ sucrose+ starch	0.072 ^b		0.335 ^b		0.998

Conclusions Results of the present study showed that the first order fractional rate of digestion of a feed might be influenced by source of NFC. In addition, there was different response in fractional rate constant of digestion and indigestible fraction when different feeds were incubated with sucrose, starch or a 1:1 mixture of starch and sucrose. Therefore, there is a need to determine the associated effect of a feed and nature of NFC on fractional rate constant of digestion and indigestible fraction for individual feed or a composed diet.

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Synthesis of ligninolytic enzymes from solid and aqueous growth of white-rot fungi on wheat straw

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Introduction There is an increased demand for raw materials that can be used as substrate for bio-ethanol production. The resultant by-products will have an important role in animal nutrition as possible feeds. Most of these potential feeds are high in cell walls with high lignin contents, limiting its nutritive value. The development and use of alternative enzyme methodologies can increase the availability of structural carbohydrates. Enzymes with the potential to break down cell walls, including lignin, are available in aerobic white-rot fungi. The aim of this study was to evaluate two white-rot cultivation procedures in wheat straw in order to determine if any differences were obtained between the ligninolytic enzyme concentrations. In addition, the susceptibility of the residual carbohydrates to hydrolysis with a commercially available cellulase was also evaluated.

Materials and methods Ten fungal strains were used to produce the enzymatic extracts, three strains of *Trametes versicolor* (TV1, TV2, TV3), *Bjerkandera adusta* (BA), *Fomes fomentarium* (FF), *Ganoderma applanatum* (GA), *Irpex lacteus* (IL), *Lepista nuda* (LN), *Phanerochaete chrysosporium* (PC), and an unknown basidiomycet (EUC). Fungi were isolated as described by Rodrigues *et al.* (2007). Enzyme extracts were obtained from cultures containing 15 g of wheat straw with 45 ml of H₂O and 0.3 ml of a mineral solution, for the solid medium, and 4.5 g of wheat straw and 100 ml of citrate buffer 50mM and 1 ml of a mineral solution, for the aqueous medium. All media were adjusted to pH 5.0. Wheat straws from the fungi growth were submitted to cellulolytic hydrolysis with Onozuka R-10. This hydrolysis was processed to evaluate wheat straw cellulose accessibility of Onozuka R-10 cellulases. Incubations, enzyme activities and the released reducing sugars were determined as described by Rodrigues *et al.* (2007). Data were analysed using one-way Anova.

Table 1 Ligninolytic activities (U/ml) of fungi cultivated on solid and aqueous culture media.

Enzyme activities	Manganese peroxidase		Lignin peroxidase		Laccase	
	Solid	Aqueous	Solid	Aqueous	Solid	Aqueous
	BA	1,27	0,01	0,11	0,00	0,00
TV1	0,34	0,14	0,01	0,00	0,04	0,18
TV2	0,20	0,06	0,04	0,00	0,03	0,15
TV3	0,90	0,08	0,00	0,00	0,05	0,07
FF	0,19	0,00	0,00	0,00	0,02	0,01
PC	0,64	0,09	0,00	0,00	0,00	0,00
EUC	0,22	0,08	0,03	0,00	0,02	0,21
LN	0,06	0,08	0,00	0,00	0,17	0,20
GA	0,25	0,08	0,00	0,00	0,07	0,03
IL	0,87	0,03	0,01	0,00	0,00	0,00

Results The enzyme activities of fungi were quite different. All together the highest enzyme activities were detected in the solid medium, with the exception of Laccase.

In the solid growth medium (Table 1) all fungi, with the exception of LN, showed high manganese-peroxidase activity, with relevant values in BA. In the same medium an expressive activity of lignin-peroxidase was only found for BA.

For almost all fungal strains laccase activity was higher in the aqueous medium with relatively high values for TV1, TV2, EUC and LN (Table 1).

There was a higher increase ($P < 0.05$) in the amount of reducing sugars of wheat straw residues (Figure 1) isolated from the solid medium incubations.

Wheat straw residues of the solid growth trial from TV1, TV2, TV3, EUC, GA and IL released a higher amount ($P < 0.05$) of reducing sugars than the control, and IL, EUC and PC also showed higher values ($P < 0.05$) than the control, for the aqueous culture medium.

However, the data seem to indicate that other enzymes might be involved in the process of facilitating enzyme access to the cellulose molecules.

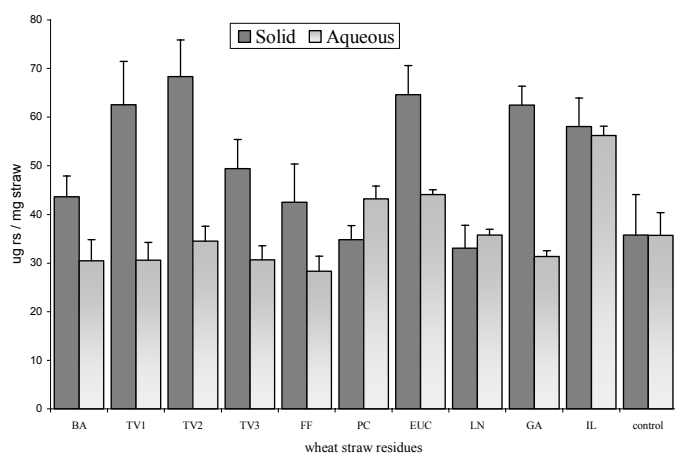


Figure 1 Reducing sugars (rs) released after incubation with a commercial cellulase.

Conclusions There were considerable differences in enzyme activities in fungi, grown in a solid and an aqueous medium. These differences do not seem to have an effect on the enzymatic degradation of cellulose.

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Effect of particle size and micro-organism on lactic acid fermentation of sorghum

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Introduction Due to the practical advantages of fermenting the carbohydrate-rich cereal component of fermented feeds separately and combining it with the protein-rich components just before feeding (Beal *et al.*, 2005; Moran *et al.*, 2006), it is desirable to have a high lactic acid concentration in the fermented cereal component so as to minimise the dilution effect on the acid concentration and pH of the feed when mixed with the protein-rich component at feeding. With the same cereal substrate and lactic acid bacteria (LAB), a key factor that might influence lactic acid production is the particle sizes produced at milling which could affect the amount of sugars available for microbial enzymatic fermentation. Further more, Anguita *et al.* (2006) indicated that technological processing of ingredients promotes higher starch hydrolysis in addition to increasing the amount of soluble non-starch polysaccharides and modifications in the physicochemical properties depending on the nature of the feed ingredients. The present study was designed to investigate the effect of particle size on fermentation of sorghum for poultry feed.

Materials and methods Sorghum was hammer-milled (3 mm screen) to provide coarse, medium, fine and very fine particles sizes. The particle sizes were separated using a Retisch flask shaker and sieve stack diameter 200 mm with apertures of 2500, 850 and 500 μm , ending in a pan for the very fine particles. The same quantities of samples (185 ± 10 g) were sieved for 10 minutes at an amplitude of 80 (vibration height = 2.4 mm) Samples were irradiated (25 kGy from Co60) in 100 g sachets before fermentation. The experiment was carried out in a 3 factor factorial experimental design with three replicates per treatment and particle size (coarse, medium, fine and very fine) as factor 1, Lactic acid bacteria (LAB)inoculants (*Lactobacillus plantarum* or *Pediococcus acidilactici* or control with no LAB) as factor 2 and fermentation time (0, 4, 8 and 24h) as factor 3. Samples were mixed with water at a ratio of 1:1.4; inoculated with 0.1 ml de Man, Regosa, and Sharpe (MRS) broth containing a 24 h culture of one of the two LABs (providing a concentration of $\sim 10^6$ colony forming units g^{-1} of feed) and incubated at 30°C. Samples were collected after 0, 4, 8 and 24 h of fermentation. Fermented samples were analysed for sugar and organic acid concentration by HPLC according to the method of Niven *et al.* (2004). Data were analysed by the general linear model procedure of analysis of variance using Minitab (release 14.0). Differences between means were determined using Tukey's test while probability values ≤ 0.05 were considered to be statistically significant.

Results The apparent rates of lactic acid production for the LAB treatments for the respective particle sizes (coarse, medium, fine and very fine) for the period between 8 and 24 hours fermentation were 12.77, 10.57, 11.12 and 13.89 mM lactic acid/h for *P. acidilactici*. Corresponding values for *L. plantarum* were 15.84, 12.25, 19.14 and 23.11 mM lactic acid/h. Results showed that it was possible with the very fine particle sizes to obtain a threshold lactic acid concentration of 110mM in the fermented cereal after 12 hours of fermentation for *L. plantarum* and 10 hours for *P. acidilactici*. The difference between the fermenting microbes in lactic acid production (Figure 1) was not significant; however, for acetic acid fermentation *P. acidilactici* had a significantly higher fermentation concentration than *L. plantarum* (Figure 2).

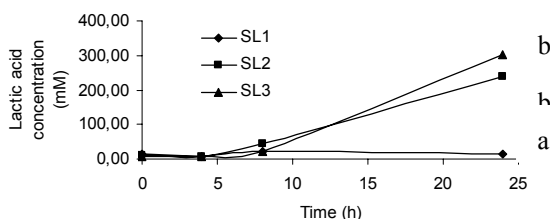


Figure 1: Variation in lactic acid concentration with incubation time (h) for sorghum fermented with *P. acidilactici* and *L. plantarum*.

Control (SL1), *P. acidilactici* (SL2), *L. plantarum* (SL3). Lines bearing different letters (a,b) differ significantly ($P < 0.0001$, S.e.d=12.22).

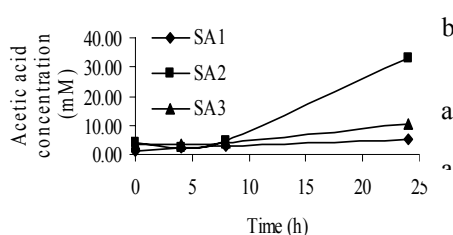


Figure 2: Variation in acetic acid concentration with incubation time (h) for sorghum fermented with *P. acidilactici* and *L. plantarum*.

Control (SA1), *P. acidilactici* (SA2), *L. plantarum* (SA3). Lines bearing different letters (a,b) differ significantly ($P = 0.0002$, S.e.d=1.922).

Conclusion

Results of this study suggest that particle sizes of sorghum influences the lactic acid produced from the grain and that *L. plantarum* is a better homofermentive option for sorghum than *P. acidilactici*.

Acknowledgement The commonwealth scholarship commission is gratefully acknowledge for financial support.

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Upgrading the *in vitro* degradability of wheat straw by using soaking treatments

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Introduction Many methods have been tested in the past for upgrading low quality roughages; physical, chemical and biological methods. Pre-soaking of compound feed pellets has been reported to have a large effect on the fermentability characteristics of this feed (Van Laar *et al.*, 2007). The application of soaking may also improve the degradation and so utilisation of fibrous feeds including cereal straws. Therefore, the objectives of the current study were to test the effect of different physical pre-treatments (soaking with water, with or without heating at different times) on the *in-vitro* dry matter degradability (DMD) of wheat straw.

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

Results The results for the main effects on DM degradation of un-soaked and soaked wheat straw are presented in Table 1. While DMD was improved with increasing soaking levels at both incubation times ($P < 0.001$), the increase in temperature reduced DMD at 46h ($P < 0.05$) but not at 92h and increase in soaking time did not show any change in DMD at any of these incubation times ($P > 0.05$). Predictably, longer incubation time of 92h showed substantially greater DMD for all treatments.

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

Treatment level	Soaking		Temperature		Time	
	46h	92h	46h	92h	46h	92h
1	402.5 ^b	513.6 ^b	422.0 ^a	536	419	531
2	410.5 ^b	535.8 ^a	413.6 ^b	532	417	537
3	440.8 ^a	553.7 ^a	NA	NA	NA	NA
SEM	3.58	4.55	2.93	3.71	2.93	3.71
Significance	***	***	*	NS	NS	NS

Values with different superscripts in the same column indicate significance at $P < 0.001$ and $P < 0.05$; SEM= standard error of means; NS= non significant; NA= Not applicable; Treatment levels= 1, 2 and 3 represent soaking levels of 1, 2 & 3 respectively, or Levels 1 & 2 represent either 20 and 60 °C or 2 and 16h respectively

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

Acknowledgement Mr. Shirif thanks Al-Fateh University and Libyan Government for the PhD scholarship

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Beef carcass composition assessed by X-ray computed tomography scanning of primal joints

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Introduction X-ray computed tomography (CT) measurements of live sheep have been used to predict carcass composition very accurately (Macfarlane *et al.*, 2006). The utilisation of spiral CT scans (SCTS) for quantifying muscle volumes and weights, using automatic image analysis procedures has also been shown to be very accurate in sheep (Navajas *et al.*, 2006). Although the limiting size of the CT gantry prevents CT scanning of live beef cattle, beef primal joints are small enough to be scanned. Hence, SCTS could be used to quantify beef carcass composition, and provide valuable information for breeding programmes including composition faster than by anatomical dissection. The objective of this study was to develop a CT image analysis procedure to assess fat, muscle and bone weights of beef carcasses and to evaluate its accuracy.

Materials and methods Data used in this study were recorded on 7 Aberdeen Angus (AAx) and 15 Limousin (LIMx) crossbred steers that were slaughtered in 2006 (average carcass weight: AAx, 354 kg; LIMx, 360 kg). The left carcass sides were split into 20 primals 48 hours after slaughter and vacuum packed. Individual SCTS of each primal, containing contiguous cross-sectional images (slices) which were 8 mm thick, were collected. Later, primals were fully dissected into fat, muscle and bone. The first step for the image analysis was to estimate the thresholds of CT density for each tissue based on the frequency distribution of pixel values in all primals, in the range -256 HU upwards, and their dissection data. The estimated thresholds were those that minimised the prediction errors. The weights of muscle, fat and bone were calculated based on tissue areas and densities and the thickness of slices in the SCTS: Tissue weight = Σ tissue area x slice thickness x weighted average density of tissue, where weighted average density = Σ (area x tissue density) / Σ area. Tissue densities were calculated for fat and muscle using the equation: Tissue density (g/cm^3) = (CT tissue density in HU x 0.00106) + 1.00062 (Fullerton, 1980). Bone density used in these calculations was 1.55 g/cm^3 . Carcass composition was calculated by adding the tissue weights of all primals measured by CT or dissection. CT and dissection weights of fat, muscle and bone of primals and carcasses were compared using linear regression analysis (model $y = \beta x$) with an intercept of zero because predictions were derived assuming a 1:1 association between CT (y) and dissection values (x).

Results The associations between the predicted composition of beef primals and their dissection values, and the accuracy (R^2) are presented in Table 1. Estimated regression slopes for the three tissues ranged between 0.99 and 1.01 and were not significantly different from one ($P > 0.05$) for fat and bone. The R^2 values for the primal composition were high (0.91 to 0.99). They are similar to the accuracy of predicting sheep carcass composition from *in vivo* CT reference scans (Macfarlane *et al.*, 2006) and SCTS (Navajas *et al.*, 2006).

Table 1 Beef primals composition: associations between CT and dissection

Tissue	Estimated slope (\pm s.e.)	R^2	r.s.d
Fat	0.997 ± 0.006	0.91	0.26
Muscle	1.007 ± 0.003	0.99	0.32
Bone	0.994 ± 0.004	0.96	0.16

s.e.: standard error of estimates; r.s.d.: residual standard deviation

The total carcass composition was also predicted with high accuracy by CT (Figure 1). In the case of carcass bone weight, the R^2 value was 0.96. The tissue thresholds estimated in this study will be independently validated in other datasets.

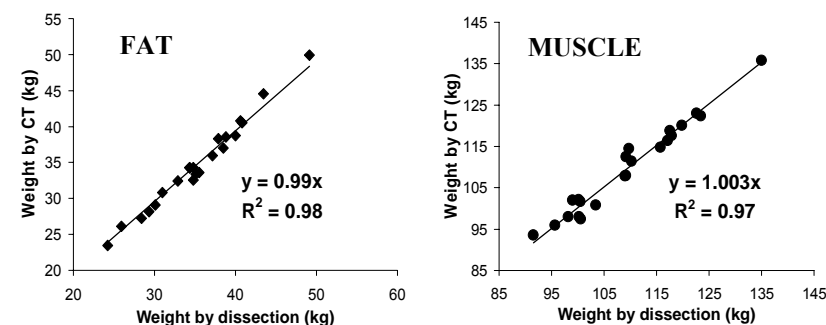


Figure 1 Total fat and muscle weights of beef carcasses by dissection and predicted by CT

Conclusions Results of this study suggest that it is possible to quantify the weights of fat, muscle and bone of beef primals and of the carcass, with high accuracy, using CT. Procedures developed for image analysis and data management allow fast processing of a significant number of carcasses. This technique may reduce costs and time of assessing carcass composition in a large number of animals, as required for the estimation of genetic parameters and in breeding programmes, including progeny tests for carcass quality.

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Repeatability and reproducibility of taste panel measurements on the meat from a commercial population of beef cattle

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Introduction The most important element in consumer enjoyment of meat is tenderness, closely followed by flavour, with variation in these traits having a significant impact on consumer satisfaction (Casas *et al.* 2006). In order to forge improvements in these traits, either through quantitative breeding or molecular genetics, it is essential that there is a reliable method of trait testing. Sensory data, as collected using a taste panel, are subject to high measurement error as well as problems associated with subjectivity. Unfortunately these sensory panels are a necessary step in the improvement of the above mentioned beef traits, as no machine can measure the range of interacting characteristics that contribute to eating quality and palatability (Warriss 2000). This study aimed to test the repeatability and reproducibility values for seven sensory beef traits assessed by a taste panel operating in a commercial setting.

Materials and methods Commercial Aberdeen Angus beef cattle (n=443) were sourced through the Scotbeef abattoir from breeder finisher farms (n=14) and were selected to be a fair representation of the British commercial cattle breed, i.e. a mix of heifers and bullocks of varying ages (between 408 and 912 days old at slaughter). Cattle were stunned by captive bolt before being slaughtered by exsanguination and dressed for commercial use. Sirloins were vacuum packed and matured for 20-31 days following slaughter after which a 2cm wide steak was cut from the middle of the sirloin and excess fat trimmed. Two steaks were cut from one sirloin in each taste panel session, with the second steak being used as a blind repeat. Sirloin steaks were cooked to 74°C using a standard flat grill. Six panellists scored the steaks on a 1-8 scale for abnormal odour, abnormal flavour, cooked steak odour, cooked steak flavour, juiciness, tenderness and overall liking, with 1 being the lowest and 8 the highest score for the measured attribute. The taste panel members were chosen from workers at the abattoir meat processing plant. Members of staff were tested using the Triangle and Matching tests (BS7767 1993), those with higher scores were chosen for the panel (n=35). Statistical analysis of the score statistics was carried out using REML analysis. Repeatability was defined as the tendency of an individual panellist to score the same product consistently for a given attribute. Reproducibility was the tendency of different panellists to, on average, give products the same score. Panel member consistency was the tendency of individual panellists to be consistent in their own scoring scheme over time. Calculations of the statistics from variance components obtained from the REML analyses were as follows:

$$\text{Repeatability} = \frac{V_{\text{Panel Date} \cdot \text{Panel Member}}}{(V_{\text{Panel Date} \cdot \text{Panel Member}} + V_{\text{Residual}})}$$

$$\text{Reproducibility} = \frac{V_{\text{Animal}}}{(V_{\text{Animal}} + V_{\text{Panel Date} \cdot \text{Panel Member}})}$$

$$\text{Panel Member Consistency} = \frac{V_{\text{Panel Member}}}{(V_{\text{Panel Member}} + V_{\text{Panel Date} \cdot \text{Panel Member}})}$$

Results Repeatability, reproducibility and panel member consistency shown in Table 1 range from 0-1, with 1 being the most repeatable/reproducible/consistent and 0 being the least. Repeatability values are moderate, with higher values for abnormal odour and flavour. The reproducibility traits are lower, mainly so for the odour and flavour traits whilst the panel member consistency scores are generally high.

Table 1 Repeatability, Reproducibility and Panel Member Consistency results for 7 beef sensory traits

Trait Measured	Repeatability	Reproducibility	Panel member consistency
Tenderness	0.38	0.26	0.59
Juiciness	0.31	0.27	0.53
Abnormal Odour	0.58	0.01	0.98
Abnormal Flavour	0.54	0.01	0.98
Cooked Steak Odour	0.38	0.05	0.88
Cooked Steak Flavour	0.36	0.03	0.92
Overall Liking	0.40	0.10	0.79

Conclusions Few animals tested had either an abnormal flavour or odour, which is reflected in the higher repeatability values seen for these traits and indicates low within- and between-animal variation. The low reproducibility values for the odour and flavour traits indicate that variation seen in the scoring of these traits is mainly within-panel rather than between-animal. Although the reproducibility values are fairly low, the relatively higher values seen for tenderness and juiciness indicate higher between-animal variation, suggesting that these traits may be the best candidates for successful improvement using breeding programmes. The high values of the panel member consistency statistic indicate that the panel members varied little over time in their scoring between the first and last panel session (14 months apart). This confirms that an animal scored in the first panel can be accurately compared with one scored in the last panel, such that the variation observed is not likely to be due to panel members changing their scoring system over the length of the experiment.

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Development and evaluation of a real-time PCR method for determining skeletal muscle fibre type composition in sheep

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Introduction Myosin is a major contractile protein in skeletal muscle and its different isoforms reflect differences in the properties of fibres, with the major fibre types being named according to the isoform of myosin heavy chain they contain. Four major adult genes encoding isoforms of myosin heavy chain (MHC I, IIA, IIX and IIB) have been identified in skeletal muscle from a variety of species (Pette & Staron, 2000), the expression of these is a characteristic of muscle fibres. Differences in the number, size and proportions of fibres are suggested to affect growth performance and meat quality (Maltin *et al.*, 2003). Conventional myosin ATPase staining characterises individual muscle fibres as slow (MHC type I) or fast (MHC Type IIA, IIX, IIB) based on the activity of myosin ATPase. We previously demonstrated that anti-myosin isoform antibodies could be used to identify muscle fibre composition by comparing to myosin ATPase staining (Sazili *et al.* 2005). The aim of this study was to develop a quantitative PCR method for determining ovine skeletal muscle fibre composition, based upon the expression of different adult MHC isoform transcripts.

Materials and methods Using published partial length ovine cDNA sequences, real-time PCR primers and probes specific for MHC I, IIA and IIX isoforms were generated at the 5' end of the transcripts. To act as positive controls, partial length MHC isoform cDNAs were generated by Reverse Transcriptase PCR to the region recognised by the real-time PCR primers and probes. To test for cross-reactivity, each MHC isoform cDNA standard curve that was to be tested with its respective real-time PCR primers and probe, was spiked with a fixed quantity of each of the other MHC isoform cDNAs. Porcine MHC IIX and IIB cDNA sequences are identical over the region to which the IIX primers and probe were designed and therefore it is likely that they will also detect MHC IIB mRNA, if present. It is not known whether MHC IIB is present in ovine skeletal muscle, but the bovine MHC IIB isoform has only been detected in extraocular muscles (Toniolo *et al.*, 2005). The primers and probes were then used to compare MHC isoform expression in muscles known to differ in fibre composition. Snap frozen samples of *supraspinatus* (SS), *semitendinosus* (ST) and *Longissimus dorsi* (LD) were obtained from wether lambs at 65 ± 2 days of age (n=10). Total RNA was extracted (Trizol) and first strand cDNA generated using random primers, prior to real-time PCR using the MHC isoform mRNA specific primers and probes.

Results We successfully isolated partial length MHC cDNAs which were characterised as MHC I, IIA and IIX isoforms. Using each MHC isoform cDNA as a PCR template for its corresponding real-time PCR primer and probe set and adding the other MHC isoform cDNAs as competitors we found that there was no interference. Therefore the MHC isoform real-time PCR primers and probes were specific to their respective MHC isoform.

The MHC isoform specific real-time PCR primers and probes were able to detect transcripts in the muscles examined and there was a significant difference in the expression of each MHC isoform transcript across muscles (Table 1). MHC I mRNA was highest in SS muscle followed by ST then LD (p<0.001), MHC IIA mRNA was higher in LD than in SS and ST muscles (p=0.018), whereas MHC IIX/IIB was lower in SS than in ST and LD (p=0.003). These data agree with our previous studies in these sheep muscles estimating proportions of fast and slow fibres using Myosin ATPase histochemistry (Sazili *et al.* 2005).

Table 1 Expression of MHC isoform mRNA in SS, ST and *Longissimus dorsi*(LD) muscles (n=10).

	SS	ST	LD	Sed	p
%MHC I	34.7	13.4	7.3	5.00	<0.001
%MHC IIA	21.2	21.5	27.5	2.22	0.018
%MHC IIX/IIB	44.1	65.1	65.2	6.02	0.003

Conclusion The method developed allows the major adult MHC isoform transcripts to be detected independently without interference from each other and can potentially be used to observe changes in muscle fibre type composition at the molecular level.

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Video image analysis of live lambs to predict live weight, carcass composition and meat quality

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Introduction Predictors of carcass and meat quality are sought that can be measured in the live animal, preferably at a young age, on-farm, and with minimum stress to the growing animal. Several *in vivo* methods of assessing these traits indirectly in lambs have been developed, most of which are expensive and require restraint/handling of animals, which can cause stress and affect growth rate. This study investigates the use of linear dimensions obtained from video image analysis (VIA) to assess live weight, carcass composition and meat quality (MQ) in lambs of two divergent breeds, in a non-disruptive way.

Materials and methods Mixed batches of male and female Texel (TEX; n=113) and Scottish Blackface (SBF; n=103) lambs were photographed at finishing (commercial slaughter weight and condition score), after being shorn. Age ranged from 101-205d and live weight from 29-47 kg. The digital images were processed to collect a synchronised set of linear dimensions taken from 3 views: the rear - highest point of rump to hock, averaged over both sides (AvQL), width of rear end (HW); the side - shoulder height (SH), rump height (HH), body depth from shoulder (SB), body depth from point of rump (HB), body length shoulder to end of rump (BL); the top - shoulder width (W1), width at narrow point behind shoulders (W2), width at widest mid-point (W3), width at narrow point in front of rump (W4), rump width (W5), length from mid-point of W1 to mid point of W5 (L1), length from mid-point of W1 to tail (L2). Multiple linear regression was used to determine the relationships of these dimensions with conformation and MQ data collected post slaughter, including total dissected weights of muscle (DMWT) and fat (DFWT) in one carcass side, chemically extracted intramuscular fat in the loin (IMF), shear force of leg (ShFLEG) and loin (ShFLOIN) muscles, and ultimate pH in leg (pHLEG) and loin (pHLOIN) muscles. The models fitted included either age (model 1) or live weight (LWT; model 2) at VIA as a covariate, as well as sex and days between VIA and slaughter. Additionally, the best combinations of VIA dimensions (chosen by comparing residual standard deviations, Mallows Cp and adjusted R²) were included, and these models were compared to models using covariates and sex only to predict product quality traits ('No VIA').

Table 1: Adjusted R² of models for product quality traits

Model	Trait	TEX		SBF	
		No VIA	Best model	No VIA	Best model
Model 1 (incl. age)	LWT	0.36	0.84	0.34	0.67
	DMWT	0.36	0.73	0.13	0.64
	DFWT	0.27	0.54	0.23	0.69
	IMF	0.19	0.38	0.10	0.40
	pHLEG	0.02	0.20	0.04	0.13
	pHLOIN	0.03	0.22	0.07	0.09
	ShFLEG	0.17	0.19	0.01	0.10
Model 2 (incl. LWT)	DMWT	0.66	0.76	0.18	0.63
	DFWT	0.53	0.62	0.26	0.69
	IMF	0.33	0.43	0.12	0.36
	pHLEG	0.07	0.26	0.09	0.13
	pHLOIN	0.07	0.24	0.05	0.12
	ShFLEG	0.17	0.19	0	0.09
	ShFLOIN	0.30	0.31	0.01	0.18

Results Weight traits (LWT, DMWT, DFWT) were predicted with moderate to high accuracy using VIA linear dimensions, age and sex (Table 1), with some inter-breed differences. Prediction accuracies were higher for LWT and DMWT in TEX (a leaner breed) and for DFWT in SBF (a fatter breed). Moderate (IMF) to low (pH and ShF) predictions of the MQ traits were achieved in both breeds. In most traits, accuracies were more than doubled by including VIA dimensions in the model, compared to only sex and age ("No VIA", Table 1). VIA predictors explained approximately 10% additional variation in DMWT, DFWT and IMF, once LWT and sex were accounted for in TEX, and a larger percentage in SBF. In TEX, VIA linear dimensions explained more variation in ultimate pH in the leg or loin muscles than age, LWT or sex. However, only a relatively small amount of the total variation was explained by all factors. VIA had little association with ShF in the leg or loin.

Traits positively associated with LWT were HW, SB, HB, L1, W1 (in TEX), W2 and W3 (in SBF). DMWT was higher in males and positively associated with lengths, widths and SB, but negatively associated with rear height (HH, AvQL) and W3 (perhaps reflecting gut-fill). DFWT and IMF were highly correlated ($r = 0.79$ in TEX, 0.67 in SBF), were higher in females, and were also positively associated with lengths, widths and SB, and negatively associated with W3 and with depth of the hind body in SBF (AvQL, HB) and SH in TEX. The most valuable set of VIA dimensions for predicting LWT and tissue weights was that measured from the top view. Adjusted R² values for models including dimensions from only the top view were <0.1 below those for the best model (Table 1) for each trait. For most traits (except age-adjusted LWT, DMWT and pH), including only the best single VIA dimension, with sex and age or LWT, reduced adjusted R² values by <0.1.

Conclusions VIA is valuable for accurately predicting weight and composition of finished shorn lambs, in a non-disruptive manner, and substantially improves the estimation of IMF. The VIA dimensions predicting composition in the two divergent breeds reflect changes in tissue proportions and distribution that are known to occur with maturity (Hammond, 1940). One potential use for this measurement method may be to select lambs with preferred weights and composition for slaughter, using a camera above a feeder or drinker in a finishing shed.

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The associations of near infrared reflectance spectroscopy and sensory characteristics or shear force measurements of beef

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Introduction Sensory characteristics of tenderness, juiciness and flavour of beef are important criteria that affect consumer's evaluation of beef quality. Near infrared reflectance spectroscopy (NIR) may be an effective tool to predict sensory characteristics of beef meat. This method is non-destructive, fast and potentially suitable for on-line measurements. The association between NIR measurements and sensory characteristics is expected to be based on chemical (e.g. water, lipid and protein content) and physical or structural (e.g. muscle fibre characteristics) properties of the meat. Objective measurements of physical or structural characteristics can be obtained using Volodkevitch shear force or slice shear force techniques (Shackelford *et al.*, 1999). The objective of this study was to investigate the associations between eating quality traits of beef and NIR measurements.

Materials and methods Data on 44 beef cattle from rotational crossed Aberdeen Angus and Limousin sires were used in the study. Animals were slaughtered in three batches at an average liveweight and age of 618 kg and 576 days, respectively. NIR measurements were taken on the *M. longissimus thoracis* between 12th/13th ribs at 48 h post mortem. Replication of 20 measurements were taken by moving, the large (relative to the sample) scanning head around the muscle sample area so that these scans were largely overlapping the same tissue. The NIR spectrophotometer (ASD Inc., Boulders, Colorado) uses two diode detection systems from 350 to 1,000 nm and 1,000 to 1,800 nm, respectively. The spectrometer interpolates the data to produce measurements at every 1 nm, resulting in a spectrum of 1,451 data points. Sensory characteristics on 1-8 scales for tenderness (1 – extremely tough, 8 - extremely tender), juiciness (1 – extremely dry, 8 - extremely juicy) and beef flavour (1 - extremely weak, 8 – extremely strong) were assessed by a trained taste panel on meat samples aged for 14 d. Texture of meat samples was measured instrumentally as shear force, using Volodkevitch jaws (10 d aged meat) and slice shear force (14 d aged meat). Preliminary analysis showed that the median of repeated measurements was the most appropriate point estimate for the correlation analysis of meat quality traits and NIR measurements within each wavelength. As systematic effects, the breed and batch were considered in order to obtain residual correlations independent of these effects using the general linear models procedure (GLM) of SAS.

Results The GLM analysis indicates that the breed and batch effects were significant for flavour ($P < 0.05$) and Volodkevitch shear force ($P < 0.01$), respectively. For all analysed beef meat quality traits, the residual correlations with NIR measurements showed high fluctuations for wavelengths from 350 to 450 nm due to reduced detector sensitivity and should therefore not be included in the prediction spectrum (Figure 1 and 2). For tenderness the highest residual correlation (0.29, $P < 0.05$) was estimated at wavelength of 638 nm. Juiciness showed its highest correlation (0.44) with the NIR measurement at 584 nm and the correlation did not change substantially over different wavelengths. The slice shear force technique showed a significant correlation (0.30, $P < 0.05$) with the NIR measurement at 1646 nm.

Figure 1 Correlations between tenderness and NIR measurements

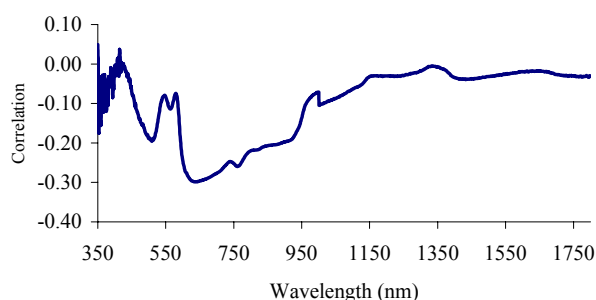
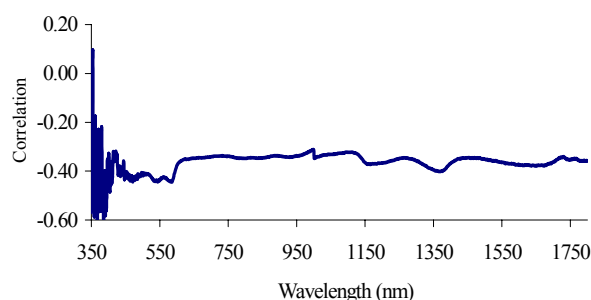


Figure 2 Correlations between juiciness and NIR measurements



Conclusions The results of the study indicate that NIR is capable of estimating sensory characteristics of tenderness and juiciness as well as texture using slice shear force technique. The highest associations of NIR measurement with tenderness were in the visible area, whereas those with juiciness were present for the entire spectrum from the visible to the infrared region. In contrast, slice shear force measurements showed its highest association with NIR in the infrared area. The results support the use of on-line NIR in the abattoir for earlier, fast and relatively inexpensive estimation of beef meat eating quality. The information could be used for quality assurance in the abattoir and potentially for genetic improvement of meat quality in breeding programmes.

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An assessment of the potential for live animal digital image analysis to predict the slaughter liveweights of finished beef cattle

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Introduction Previous work has shown that dimensional information derived from visual images can be used to accurately estimate pig growth, in terms of size and shape (Doeschl *et al*, 2004). The use of visual images to derive accurate estimates of weight could be very useful information in the commercial environment within and across the livestock industries. The objective of the current study was to examine a small number of digital images of finished beef cattle to ascertain if digital image analysis (DIA) has potential to predict the liveweight (LW) of the animals at slaughter.

Materials and methods A total of 27 digital images of finished beef cattle were obtained within three days of the animals going for slaughter from beef cattle studies conducted at SAC during 2006 where slaughter liveweights were determined. Images from ten Aberdeen Angus x Limousin and seventeen Limousin x Aberdeen Angus steers were collected. Continuous images were taken using a multiplex digital camera mounted directly above the animal and immediately adjacent to the water trough in the corner of the straw-bedded pen in which the animals were housed. Images were stored on computer and selected static images were subsequently processed to obtain simple dimensional data using image processing software (Inspector 8) by an experienced operator. Simple dimensions collected for each animal were:- body length from the tailhead to the front of the shoulders (L1), body width across the widest part of the rump (W1), body width across the narrowest part between the belly and the rump (W2), body width across the widest part of the belly (W3), body width across the narrowest part behind the shoulders (W4) and body width across the widest part of the shoulders (W5). Derived volumes were also calculated for each animal (V1, V2, V3, V4, V5 and V AveW) as cylindrical volumes (m³) using the L1 and W values respectively. Simple and multiple regression relationships between all potential basic DIA dimensions (mm) and derived volumes (m³) obtained on images of these 27 live animals and slaughter LW were established using Genstat 5.

Results The adjusted R² and residual standard deviation (R.S.D) values for the regression relationships between simple dimensional data, derived cylindrical volumes and slaughter LW are given in Table 1. The best single predictor of slaughter LW was the derived cylindrical volume using the average body width measurement (V AveW) with an R² of 0.361 and an R.S.D of 21.6. The predicted vs actual slaughter LWs using the best multiple regression relationship are shown in Figure 1.

Table 1. Adjusted R² and R.S.D of simple regression relationships between basic DIA dimensions, derived volumes and slaughter LW of finished beef cattle.

Basic dimension	R ²	R.S.D.
L1	0.085	25.9
W1	0.149	24.9
W2	0.135	13.5
W3	0.197	24.2
W4	0.117	25.4
W5	0.153	24.9
Ave W	0.274	23.0
Derived cylindrical volume		
V1	0.215	24.0
V2	0.207	24.1
V3	0.247	23.5
V4	0.220	23.9
V5	0.247	23.5
V AveW	0.361	21.6

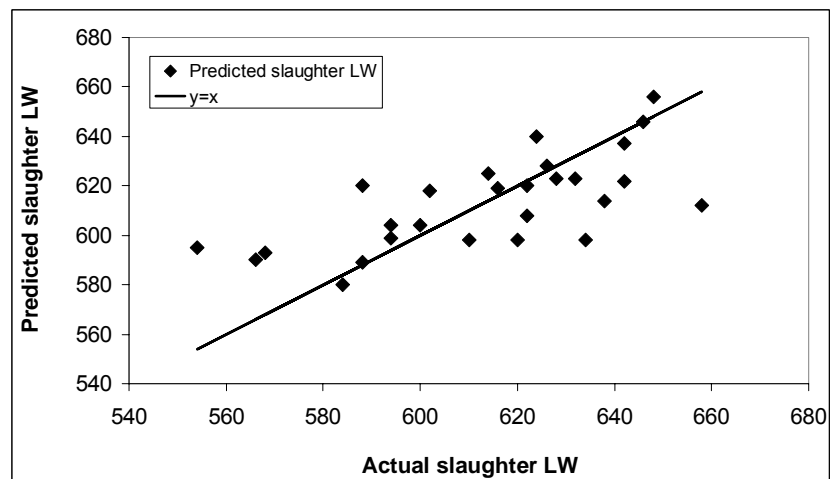


Figure 1 Predicted vs actual slaughter LWs using the best multiple regression model from basic dimensional parameters (terms included in the model were V AveW, log V2 and log W5 {R² = 0.404: R.S.D. = 20.9}).

Conclusions Although the simple dimensional variables studied here explained a limited proportion of the variation in slaughter LW in this small sample of finished beef cattle, results do indicate that dimensional data obtained from DIA has potential to predict the slaughter LW of finished beef cattle. Further studies are required to examine the feasibility of collecting DIA images over the finishing period with beef cattle housed in conditions akin to commercial practice. In addition, more robust prediction equations using a wider range of simple and derived predictor variables should also be examined for a wider range of current and future commercially important traits.

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The effects of forage: concentrate ratio on meat quality of bulls slaughtered at a range of liveweights

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Introduction The current upward trend in cereal prices will have a negative impact on the economics of intensive beef finishing systems. Patterson *et al* (1994) concluded that continental bulls could perform at a high level on diets which were relatively low in concentrates and predominantly based on grass silage. The objective of the present study was to evaluate the effect of forage:concentrate ratio and weight at slaughter on the meat quality of ¾ or more continental bulls.

Materials and methods A total of 260 (120 year 1; 140 year 2) continental bull calves (¾ or greater Charolais, Belgian Blue or Limousin breeding) with a mean initial live weight 351 ± 42.3 kg and mean age 8 ± 1.5 months were allocated to two dietary treatments balanced for breed, initial weight and age. The diets consisted of *ad libitum* concentrates supplemented with 1.2 kg silage dry matter (DM) or a totally mixed ration containing grass silage and concentrates at a 50:50 ratio, on a DM basis. Equal numbers of cattle again balanced for breed, initial weight and age were slaughtered after 191, 218 or 254 days on experiment in year 1 and 190, 217 or 253 days in year 2. Assessments of ultimate pH, sarcomere length, cooking loss, Warner Bratzler shear force and meat colour were undertaken on the *longissimus dorsi* muscle. The data were analysed using Genstat regression procedures with a model including treatment as a factor and slaughter weight as an independent variable and their interactions. Predicted values were calculated for each treatment at a range of slaughter weights.

Results The silage offered had a predicted D-value of 780 and 700 g/kg DM in years 1 and 2, respectively. Animal performance data has been presented by Keady *et al* (2006) and Dawson *et al* (2007). Slaughter weight had no significant effect on meat colour as assessed by L*, a* or b* (Table 1). There was a tendency (P=0.07) for cooking loss to decline after 7 days ageing as slaughter weight increased. Slaughter weight had no significant effect on meat quality as assessed by ultimate pH, sarcomere length or Warner Bratzler shear force. Decreasing the forage:concentrate ratio decreased (P<0.05) cooking loss assessed after 7 days ageing but had no other significant effect on meat quality (Table 2) or meat colour (Table 3).

Table 1 Effects of slaughter weight on meat colour and quality (prediction¹)

	Slaughter weight (kg)							Sem	Sig
	500	550	600	650	700	750	800		
<i>7 day ageing</i>									
Ultimate pH	5.63	5.63	5.63	5.63	5.63	5.63	5.63	0.027	NS
Sarcomere length (µm)	2.68	2.69	2.71	2.72	2.73	2.75	2.76	0.039	NS
Cooking loss (g/kg)	30.1	29.8	29.6	29.3	29.1	28.8	28.5	0.39	P=0.07
WBSF [†] (kg/cm ²)	3.47	3.48	3.48	3.49	3.50	3.50	3.51	0.088	NS
<i>21 day ageing</i>									
Cooking loss (g/kg)	30.7	30.4	30.1	29.9	29.6	29.3	29.1	0.42	NS
WBSF [†] (kg/cm ²)	3.14	3.13	3.13	3.13	3.12	3.12	3.11	0.081	NS
<i>Meat colour</i>									
L* (lightness)	40.9	40.7	40.5	40.3	40.1	39.9	39.7	0.73	NS
a* (redness)	17.1	17.2	17.2	17.3	17.4	17.4	17.5	0.39	NS
b* (yellowness)	13.8	13.9	13.9	14.0	14.0	14.0	14.1	0.29	NS

Table 2 Effect of forage:concentrate ratio on meat quality

	Forage: Concentrate ratio			Sig
	50:50	0:100	Sem	
<i>7 day ageing</i>				
Ultimate pH	5.61	5.65	0.023	NS
Sarcomere length (µm)	2.75	2.69	0.032	NS
Cooking loss (g/kg)	29.7	28.7	0.32	*
WBSF [†] (kg/cm ²)	3.43	3.55	0.074	NS
<i>21 day ageing</i>				
Cooking loss (g/kg)	30.1	29.5	0.35	NS
WBSF [†] (kg/cm ²)	3.06	3.19	0.066	NS

Table 3 Effect of forage: concentrate ratio on meat colour

	Forage: Concentrate ratio			Sig
	50:50	0:100	Sem	
<i>Meat colour</i>				
L*	39.7	40.7	0.61	NS
a*	17.1	17.5	0.32	NS
b*	14.0	13.9	0.24	NS

¹Values predicted from regression analysis

[†] WBSF Warner Bratzler Shear force

Conclusions The results of the current trial have demonstrated that continental bred bulls can be slaughtered at weights up to 800 kg live weight without detrimental affects on meat colour or quality. Replacing 0.50 of the *ad libitum* concentrate diet with grass silage resulted in an increase in cooking loss after 7 days ageing, but had no other effect on meat colour or quality.

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Meat quality of Charolais steers: influence of feeding grass versus red clover silage during winter followed by finish off grass

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Introduction We have previously demonstrated that feeding red clover relative to grass silage results in meat characterised by higher levels of polyunsaturated fatty acids (PUFA) but reduced shelf life which was associated with lower levels of vitamin E in the muscle (Scollan *et al.*, 2006). Colour shelf life could be ameliorated by feeding additional vitamin E (Scollan *et al.*, 2006). Feeding red clover silage followed by finishing off pasture may help alleviate the problem of colour shelf life while maintaining the benefit of the legume in delivering higher PUFA into meat. Hence this study examined feeding red clover compared with grass silage during the winter, following by a summer finishing period at grass, on fatty acid composition, vitamin E content of meat, colour shelf life and sensory attributes of beef.

Materials and methods Twenty seven Charolais steers (initial live weight 496 kg (s.e.d. 14.9)) were randomly allocated to one of three *ad libitum* silage treatments (each consisting of nine animals) (1) grass silage, (2) red clover or (3) 50:50 mix (dry matter basis) of grass and red clover. Following ~ 120 days of silage treatments animals were turned outdoors and rotationally grazed on perennial ryegrass pastures. Animals were slaughtered after ~ 100 days at grazing and samples of *m. longissimus thoracis et lumborum* were taken at 48h post-mortem for fatty acid analysis, vitamin E analysis and 10-day aged samples for sensory analysis, or shelf life studies in modified atmosphere packs. An ANOVA with diet as the main factor was used to analyse the data.

Results Liveweight gain was similar across the different silages (mean 1.07 kg/d) and carcass weight and conformation and fat score were also similar (mean 191 kg, 90.2 (0-150 line scale) and 73.3 (0-150 line scale)), respectively. Total muscle fatty acids and proportions of the major saturated fatty acids were not different (Table 1). The proportions of 18:2 n -6 and 18:3 n -3 were higher on red clover relative to grass silage resulting in higher polyunsaturated to saturated (P:S) ratios on the red clover. There were no differences in vitamin E content of the muscle, all being higher than the recommended 3.5 required for optimum stability, resulting in similar colour and lipid stability for all treatments. There were no differences in eating quality between treatments as assessed by a trained sensory panel.

Table 1 Fatty acid composition of *longissimus thoracis et lumborum* and colour shelf life of loin steaks during simulated retail display and vitamin E content of muscle

	Silage treatment			SED	P
	Grass	Grass/red clover (50:50)	Red clover		
Total fatty acids (mg/100g muscle)	2115	2607	1945	340.8	NS
16:0	23.7	24.7	23.5	0.81	NS
18:0	14.8	15.8	15.7	0.70	NS
18:1 n -9	31.2	32.4	30.1	1.17	NS
18:1 <i>trans</i>	2.58	2.22	2.17	0.274	NS
Conjugated linoleic acid (CLA)	0.49	0.41	0.39	0.064	NS
18:2 n -6	2.98	2.44	3.69	0.398	0.016
18:3 n -3	1.76	1.63	2.34	0.237	0.001
EPA	0.89	0.67	0.95	0.148	NS
DHA	0.17	0.12	0.22	0.064	NS
DPA	1.02	0.75	1.10	0.127	NS
P:S	0.12	0.10	0.15	0.017	0.024
<i>n</i> -6: <i>n</i> -3	1.15	1.09	1.13	0.067	NS
Vitamin E (mg/100g muscle)	4.46	3.92	4.30	0.303	NS
TBARS d10 (mg/100g muscle)	1.72	1.90	2.10	0.474	NS
Colour saturation day 9	19.06	18.46	18.96	1.22	NS

Conclusions Feeding red clover relative to grass silage followed by a period grazing fresh grass increased the content of PUFA in the meat and helpfully no differences in colour, shelf life or sensory attributes. This most likely relates to the fresh grass providing sufficient vitamin E to correct the lower amounts found with red clover feeding (Scollan *et al.*, 2006). The higher levels of PUFA in meat from red clover may relate to the action of the plant enzyme polyphenol oxidase (PPO) protecting some of the beneficial plant PUFA from the biohydrogenation in the rumen.

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Colour and lipid stability of beef from grazing or concentrate-fed cattle with and without supplemental alpha-tocopheryl acetate stored in a high oxygen atmosphere when intact or minced

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Introduction Supranutritional supplementation of cattle with vitamin E has improved the colour and lipid stability of beef. Grass-fed cattle have muscle vitamin E concentrations which are 2 to 3 times greater than unsupplemented concentrate-fed cattle (Lanari *et al.*, 2002) but also have higher concentrations of the highly oxidisable long chain n-3 polyunsaturated fatty acids (Nuernberg *et al.*, 2005). The objective was to compare the oxidative stability of grass- with concentrate-fed beef, both minced and intact, and to determine if supplemental vitamin E can increase the oxidative stability of such beef in high oxygen packaging.

Materials and methods For comparative purposes, samples of *M. longissimus dorsi* (LD) from 40 continental cross heifers were selected from a larger experiment. All heifers had been blocked according to body weight and within block, assigned randomly to one of 9 dietary treatments (n = 10 per treatment) of which, 4 treatments were selected for the present work, i.e. two outdoor treatments – a restricted grazing allowance plus 2.5kg of supplementary concentrates containing either no added vitamin E (G0) or 400 I.U. (international units) of vitamin E, as alpha-tocopheryl acetate (G1000) and two indoor treatments, receiving 70% of a basal concentrate ration plus 2.5kg of the same supplementary concentrates as the grazing heifers but with either no added vitamin E (C0) or 200 I.U. of vitamin E (C500). Following slaughter, samples of LD were excised from the 12th rib and a section was vacuum packaged and immediately frozen at -20°C. After 19 months of frozen storage, samples were thawed for 72 hours at 0°C. One half was cut into steaks and the other half was minced through a plate with 3mm holes and formed into patties. Steaks and patties were packaged in a high oxygen atmosphere (79.6%O₂:17.8%CO₂) and displayed for 10 days. Colour ('a' (redness), 'b' (yellowness), 'C' (saturation), 'H' (hue angle) and 'L' (lightness)) was measured on days 0, 1, 3, 6, 8 and 10 and lipid oxidation (as thiobarbituric acid reactive substances, TBARS) was measured on days 0, 3, 6 and 10. Vitamin E in LD was measured. Data were analysed as a split-split plot with effects of dietary treatment in the main plot, comminution (intact or minced) in the sub-plot and time (days) in the sub-sub plot and all interactions included in the model. Where significant differences were detected means were compared using Fisher's least significant difference test.

Results Vitamin E concentrations in LD muscle were 1.46, 2.32, 2.84 and 2.90µg⁻¹ for C0, C500, G0 and G1000, respectively, with no significant difference between the latter 3 treatments. There was a significant comminution × time interaction for all colour variables (P<0.001 for 'L', 'a', 'C', 'H' and P=0.007 for 'b') and for TBARS (P<0.001). Minced LD was initially more red (P<0.05) on days 0, 1 and 3 but less red (P<0.05) on day 8 than intact LD. There was a treatment × time interaction for 'a', 'H' (both P<0.001), 'C' (P=0.029) and TBARS (P<0.001). The C0 LD was less red (P<0.05) on day 3 and tended to be less red thereafter. The G0 LD tended to be most red on all days and both G treatments were more red (P<0.05) on days 6, 8 and 10. Minced LD had higher TBARS on day 0 (P<0.05) and days 3, 6 and 10 (all P<0.001) than intact LD. The G1000 LD had the lowest (P<0.05) TBARS on day 3 and 10 while the G treatments tended to have lower TBARS on days 6 and 10.

Conclusion It is concluded that while increased vitamin E stabilised lipids (indicated by lower TBARS values) it is not required to stabilise colour of grass-fed beef and that mincing causes increased discolouration and lipid oxidation.

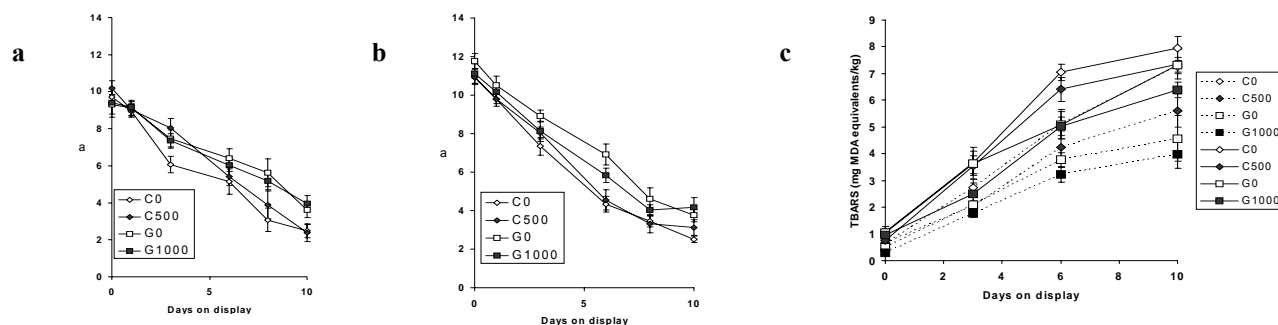


Figure 1 Change in redness of (a) intact and (b) minced LD muscle; (c) thiobarbituric acid reactive substances (TBARS) of intact (broken lines) and minced (solid lines) LD muscle.

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Comparison of different hanging methods of lamb carcasses

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Introduction Tenderness can be considered as a function of three components: connective tissue content/composition, sarcomere length and proteolysis of the myofibrillar proteins (ageing) (Koochmaraie, 2002). Improvement of sarcomere length and proteolysis can be achieved through optimal processing (e.g. hanging and aging; Thompson, 2006). The main technique that improves the sarcomere length is tenderstretch hanging. This technique increases the tension of the hindlimb and loin muscles avoiding the contraction of the fibers at rigor (Bouton, 1973). In this experiment the aim was, under commercial conditions, to compare two methods of tenderstretch hanging and to examine the potential to improve the tenderness of lamb muscles.

Materials and methods The work was conducted using three experimental groups; the lamb carcasses of the first group were hung from a frame with hooks that supported six carcasses from the Achilles as is normal practice in the meat plant. The carcasses of the second group (Tenderstretch 1) were suspended from the *pubis symphysis* by the same frame with six points hook, and the third group (Tenderstretch 2) were suspended by a single hook placed under the *pubis symphysis* (Thompson *et al.*, 2005), and then attached to the six point frame. The single hook used was a tenderstretch hook for beef carcasses. The carcasses were placed in a chill room for 72 hours. Samples for sarcomere length were collected, two from the loin (*longissimus thoracis*, LT and *longissimus lumborum*, LL) and one from leg (*Biceps femoris*, BF). At day 10 post slaughter, the LL were dissected out, placed in vacuum packs and cooked in a water bath (50 minutes at 75°C). Warner Bratzler shear force (WBSF) was measured on six 1.3 cm diameter cores from each sample (LL). The same preparation was conducted on the leg muscles on day 12: *Rectus femoris* (RF), *Vastus lateralis* (VL), *Abductor* (AD) and *Biceps femoris* (BF) and WBSF was measured with a minimum of four cores from smaller muscles (RF and VL). Sarcomere length was measured by a Helium-Neon laser diffraction technique and WBSF shear force by an Instron texture analyzer. The results were analyzed by Analysis of Variance using Genstat.

Results There were statistically significant differences in the sarcomere length between both tenderstretch methods and achilles suspension for *longissimus thoracis*, *longissimus lumborum* and *Biceps femoris*. Between the two methods of hip suspension, there was a statistically significant difference in sarcomere length only for the sample from *longissimus thoracis* area (Table 1). The WBSF values for the LL were significantly higher ($P < 0.001$) for the Achilles suspension than the tenderstretch methods. There were no differences in WBSF between the two tenderstretch methods for the LL. There were no statistically significant differences in WBSF between the three methods of suspension for any of the leg muscles (RF, VL, AD, and BF) analyzed (Table 1).

Table 1 Effect of hanging method on Warner Bratzler Shear Force and Sarcomere length for muscles of lambs selected

Hanging Method	Warner Bratzler Shear Force (WBSF) (kgf.cm ⁻²)					Sarcomere length (µm)		
	LL	RF	VL	AD	BF	LT	LL	BF
Achilles susp.	2.41 ^a	2.01	2.13	2.47	2.22	1.78 ^a	1.75	1.81
Tenderstretch 1	1.85 ^b	1.91	2.18	2.55	2.29	1.99 ^b	2.06	2.96
Tenderstretch 2	2.00 ^b	2.02	2.28	2.51	2.44	2.10 ^c	2.14	2.84
Significance	***	ns	ns	ns	ns	***	***	***
l.s.d.	0.27	0.20	0.22	0.24	0.22	0.10	0.15	0.15

ns indicate not statistically significant ($P > 0.05$); *** $P < 0.001$; lsd least significant difference; within a column means with common superscripts are not significantly different.

Conclusions The results of this experiment suggest that both tenderstretch hanging methods used for the lamb carcasses had a beneficial effect on the tenderness of the loin, as measured by the Warner Bratzler shear force technique and sarcomere length. These results show that hip suspension by the six point hook normally used in some plants, could be an effective and cheaper method to tenderstretch lamb carcasses, despite these are in contact with adjacent carcasses.

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Effect of dietary concentration of docosahexaenoic acid on enrichment of organ tissues of the chicken with docosahexaenoic acid

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Introduction Human intakes of Very Long Chain *n*-3 Polyunsaturated Fatty Acids (VLC *n*-3 PUFAs) are sub-optimal and enrichment of animal-derived foods with C22:6 (DHA) provides a potential route to increased intakes. Chicken meat is popular and versatile, and has the potential to provide meaningful amounts of VLC *n*-3 PUFAs to the human diet if enriched with these fatty acids through supplementation of growing broiler diets. Marine oils are potential sources as they are known to be rich in DHA. The enrichment of edible poultry tissues with DHA, particularly breast muscle is well established. However, the effect of dietary supplementation of DHA, on the enrichment of other tissues of the chicken is not well known, therefore work to investigate the distribution of these fatty acids within the broiler chicken is valuable to further understand the distribution and enrichment process. The objective of the present study was to compare different levels of DHA in broiler diets in relation to its effect on DHA concentration in the major organ tissues of the broiler chicken.

Materials and methods Day old, mixed sex, Ross 308 chicks (144) were reared together in a single group and fed on a proprietary chick crumb for three weeks. At age three weeks, the birds were randomly allocated to one of six experimental groups. Six birds were allocated to a cage and there were four cages per diet. The birds were fed diets supplemented with either soya oil (control) or one of five diets containing three different levels of DHA from marine sources. The experimental diets were formulated to contain 2.5, 5 or 7.5 g DHA/kg of feed. At six weeks of age the birds were humanely slaughtered and two birds from each pen were dissected. Samples of brain, liver, kidney, heart, skin, abdominal fat, breast and leg tissues were harvested and immediately frozen pending analysis. Diets and all tissues were analysed for fatty acids by gas chromatography- mass spectrometry. A regression analysis of dietary DHA concentration against tissue DHA concentration were performed.

Results The results of the regression analysis of dietary DHA and DHA in each tissue are summarised in Table 1. Increased DHA concentrations were found in all tissues in response to increasing dietary supply of DHA but different tissues contained different levels of DHA. The regression results indicate that brain tissue yielded the greatest concentration of DHA whilst breast and liver tissue showed the greatest response to increased dietary DHA. Kidney, skin, abdominal fat, heart and leg tissues yielded low levels of DHA although a small increase was seen in response to increased DHA in the diet.

Table 1 Regression statistics for dietary DHA against tissue DHA

Tissue	Regression Statistics						
	Coefficient	SE coefficient	Constant	SE constant	R ²	S	P
Breast	2.73	1.174	1.58	0.288	0.883	1.47	0.005
Leg	1.16	0.320	0.54	0.078	0.922	0.40	0.002
Liver	4.96	1.661	1.14	0.407	0.661	2.08	0.049
Kidney	1.16	0.710	0.588	0.174	0.741	0.89	0.028
Skin	-0.012	0.237	0.550	0.058	0.957	0.297	0.001
Abdominal fat	0.065	0.314	0.521	0.077	0.920	0.393	0.002
Brain	14.1	1.051	0.610	0.257	0.584	1.32	0.077
Heart	0.285	0.208	0.421	0.051	0.945	0.261	0.001

Conclusions These data indicate that the chicken brain appears to have a particular preference for DHA and yields higher concentrations than other organs when increased concentrations of DHA are provided in the diet. The liver also shows a large initial response to increased supply of DHA indicating a preference for DHA partitioning into this tissue. Unlike the brain, the low concentrations of DHA in the skin, kidney, heart and abdominal fat indicate that these organs are not a priority in the deposition process. The breast muscle is also particularly responsive to increased supply of DHA in the diet. This therefore means the viability of the enrichment of this important edible tissue, in efforts to increase supply of DHA to the human diet, is valuable.

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Effect of different inclusion rates of fish oil and vitamin E in broiler diets on the long chain n-3 polyunsaturated fatty acid content and sensory analysis of chicken meat

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Introduction Enriching chicken meat with the long chain n-3 polyunsaturated fatty acids (LC n-3 PUFA) 20:5 (EPA) and 22:6 (DHA) by the inclusion of fish oil in the poultry diet is a means of increasing the human consumption of these essential fatty acids. However, a potential drawback of this practice is the adverse effect this can have on the sensory characteristics of chicken meat. Although freshly cooked meat may still be acceptable, oxidative stability of cooked and refrigerated meat from birds fed diets containing more than 40 g/kg fishmeal can be greatly diminished due to the development of unacceptable flavours in the cooked meat when it is reheated (O'Keefe *et al.*, 1995). However, increasing the vitamin E content of the poultry diet increases the oxidative stability of the meat (Nam *et al.*, 1997). The objective of this experiment was to determine the effects on LC n-3 PUFA content and sensory characteristics of white chicken meat when the inclusion rates of vitamin E and fish oil were increased in broiler diets.

Methods Day old, mixed sex, Ross 308 chicks (144) were reared together as a single group for three weeks on a proprietary chick crumb (CWF Chick Crumbs ACS, Countrywide Farmers, Worcestershire, UK), before being randomly allocated to one of six experimental groups. Six birds were allocated to a cage and there were four cages per diet. The birds were fed diets supplemented with vitamin E (α -tocopherol) and either 40 g/kg blended vegetable oil (control, CON) or fish oil. Fish oil was included at rates of 40, 60 or 80 g/kg diet (denoted 4F, 6F or 8F). Vitamin E was included at rates of 100 iu/kg in CON, and at rates of 100, 150 or 200 iu/kg in the fish oil containing diets (denoted 100, 150 or 200). The six experimental diets were CON, 4F100, 6F100, 6F150, 8F100 and 8F200. Birds were fed these diets for 21 d, before being humanely slaughtered at 42 d. Samples of skinless white meat were taken from two birds in each cage and analysed for fatty acids by gas chromatography-mass spectrometry. Skinless white meat from all other birds was bulked by treatment, cut into cubes (20 g) and frozen for two weeks. Samples were thawed, cooked (180°C, 20 min, wrapped in aluminium foil), refrigerated overnight at 4°C, reheated to 60°C and assessed by sensory analysis with panels of 60 trained volunteers using the R index test (Brown, 1974) to determine whether samples were significantly different in taste and texture from the reference (CON) sample. The effect of diet on the fatty acid composition of the meat was assessed by analysis of variance using Mintab 15.

Results The results are summarised in Table 1. Increasing the inclusion rate of fish oil in the diet increased both the EPA and DHA content of the meat. A 200 g serving of these meats would provide mean EPA+DHA consumptions of 44, 260, 332 and 418 mg for CON, 4F, 6F and 8F respectively. Meat from birds fed 100 iu vitamin E/kg diet was acceptable when the inclusion rate of fish oil was 40 g/kg but not 60 or 80 g/kg. Increasing the vitamin E content in proportion to the increased inclusion rate of fish oil did not prevent the meat from birds fed 60 g/kg fish oil being unacceptable, but 200 iu vitamin E/kg was sufficient for the meat from birds fed 80 g/kg fish oil. Panellists who correctly identified samples as being different from the reference sample were asked to describe the differences they detected, and most reported fishy tastes in the meat from birds fed fish oil.

Table 1 Effect of poultry diet on meat fatty acid concentration (mg/100 g fresh tissue) and perception of difference (R index, %) from reference (CON) sample.

Fatty acid	Diet						sem	P
	CON	4F100	6F100	6F150	8F100	8F200		
EPA	6.05	32.8	40.9	35.0	49.8	49.8	3.57	<0.001
DHA	15.5	97.4	129	126	165	153	7.43	<0.001
R index	-	57.8	63.2*	64.3*	66.5*	57.3		

*denotes samples that were calculated to be significantly ($P < 0.05$) different from the reference (CON) sample in terms of taste and texture.

Conclusions Poultry meat can be enriched with LC n-3 PUFA without affecting the sensory characteristics of the meat if high concentrations of vitamin E are included in the diet. Including fish oil at 40 g/kg diet is satisfactory with 100 iu vitamin E/kg diet. Higher inclusion rates of fish oil require greater than proportional increases in the vitamin E content of the diet, with 200 iu vitamin E/kg diet required to maintain the acceptability of meat enriched with 200 mg LC n-3 PUFA/100 g fresh meat.

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Comparison of fatty acid composition of organic grazed lambs with lambs finished by grazing or indoor intensive concentrate feeding

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Introduction The United Kingdom Food Standards Agency (FSA, 2007) noted that the balance of current scientific evidence did not support the view that there were nutritional benefits in consuming organically produced food, although they acknowledged environmental and animal welfare benefits. Nurenberg *et al.* (2005) reported an increase in *n*-3 polyunsaturated fatty acids (PUFA) and conjugated linoleic acid (CLA *cis*-9, *trans*-11) levels in muscle and subcutaneous fat from lambs grazing fresh pasture compared to concentrate-fed lambs. In this report, fatty acid composition data from two lamb finishing trials were statistically analysed to compare the effects of organic grazing with grazing or indoor intensive concentrate feeding.

Materials and methods Lleyn-sired entire male lambs (*n*=21) were produced under an organic system grazing grass/red clover swards initially and finished on grass/white clover swards (no concentrates). Animals were slaughtered in mid-late October at 44-51 kg live weight. As part of a separate trial, Texel and Lleyn-sired entire male lambs were finished by grazing (66.3 days) mixed perennial and Italian ryegrass pastures (*n*=8) or intensively finished indoors (62.5 days) on a barley/soya-based concentrate (167 g crude protein/kg dry matter) offered *ad libitum* with 30g/day chopped straw (*n*=12). Animals were slaughtered through November/December at two live weights, 42 and 50 kg. A loin chop (*Longissimus dorsi*) from each carcass was dissected into adipose and muscle tissue, homogenised separately and the lipid extracted using chloroform:methanol. Fatty acid methyl esters were prepared and analysed by capillary column gas-liquid chromatography. One-way ANOVA with contrast was applied to compare results from each trial and dietary treatment.

Results Fatty acid data was expressed as mg/g of tissue hence the lower fat contents in muscle tissue were reflected in lower concentrations of fatty acids than were determined in adipose tissue (Table 1). Dietary treatment had a significant (*P*<0.001) effect on C18:2 levels (main *n*-6 PUFA determined) in the adipose and muscle tissues, with intensive concentrate feeding resulting in higher (*P*<0.05) levels than organic grazing which gave higher (*P*<0.05) levels than grazing. Although dietary treatment had no significant effect (*P*>0.05) on C18:3 levels (main *n*-3 PUFA determined) in the muscle tissue there was a significant (*P*<0.01) effect on levels in the adipose tissue, with organic grazed lamb having higher levels (*P*<0.05) of this acid than the grazed or intensive concentrate-fed lambs, which were not significantly different (*P*>0.05). The *n*-6:*n*-3 ratio in muscle tissue of organic grazed lambs was significantly lower (*P*<0.05) than the grazed or intensively-finished concentrate-fed lambs (not significantly different *P*>0.05), but grazed lambs had a lower ratio (*P*<0.05) in adipose tissue than organic grazed which was lower (*P*<0.05) than the intensive concentrate-fed lambs.

Table 1 Fatty acid composition of organic grazed lambs, grazed lambs and indoors intensive concentrate-fed lambs

Fatty acid	mg fatty acid/g of tissue						Statistical significance			
	Organic grazed		Grazed		Indoor intensive		adipose SED	P	muscle SED	P
	adipose	muscle	adipose	muscle	adipose	muscle				
C18:2 <i>n</i> -6	15.94	1.02	6.08	1.29	20.36	1.98	3.057	<0.001	0.211	<0.001
C20:4 <i>n</i> -6	2.13	0.12	0.35	0.11	0.66	0.10	0.367	<0.001	0.028	NS
C18:3 <i>n</i> -3	7.66	0.66	4.71	0.66	4.38	0.66	1.059	0.01	0.100	NS
C20:5 <i>n</i> -3	0.91	0.13	0.00	0.09	0.29	0.15	0.339	0.017	0.027	NS
C22:6 <i>n</i> -3	0.36	0.03	0.00	0.03	0.11	0.03	0.206	NS	0.016	NS
<i>n</i> -6: <i>n</i> -3 ratio [†]	2.36	1.41	1.36	2.59	4.92	2.61	0.725	<0.001	0.533	0.019
Total SFA	359.1	19.60	320.9	29.10	304.0	36.90	18.840	0.005	3.770	<0.001
Total USFA	337.6	21.90	269.4	29.50	306.4	38.30	16.500	<0.001	3.440	<0.001
Total CLA	6.81	0.49	6.94	0.70	4.46	0.89	1.527	0.002	0.289	0.010

[†] Calculated for each sample individually for statistical analysis

Conclusions The effects of the dietary treatments on lamb fatty acid composition may have been confounded by several factors; (i) the organic grazed lambs were not from the same genetic pool as those finished by grazing or intensive concentrate feeding, and (ii) the grazed lambs were finished on late autumn pasture that may have been of poor quality due to adverse weather conditions. Fatty acid results were expressed as mg/g tissue to inform the consumer but this format is influenced by tissue fat content and hence comparisons of the effect of dietary treatments are not straightforward. From the data analysed, there is no overwhelming evidence to suggest that lamb produced by organic grazing had improved potential health benefits even though the *n*-6:*n*-3 PUFA ratio of the muscle tissue of organic grazed lambs was lower than the grass or intensively-finished concentrate-fed lambs. All treatments however resulted in ratios below the recommended value of 5 (Lunn and Theobald, 2006). Higher CLA values in the muscle tissue of intensively finished lambs are not easily explained.

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Effect of finishing system and fish oil supplementation on the fatty acid composition of muscle from late season lamb

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Introduction It is widely accepted that the intake of *n*-3 polyunsaturated fatty acids (PUFAs) in the human diet should increase, in particular the long chain *n*-3 PUFAs such as docosahexaenoic acid (DHA, 22:6*n*-3). Diet has a significant effect on the fatty acid profile of lamb muscle. Meat from concentrate-fed lambs generally has the highest ratio of *n*-6:*n*-3 PUFAs, although supplementation with fish oil has been used to enrich the 22:6*n*-3 content of lamb (Wachira *et al.*, 2002). Lambs finished off grass, a rich source of α -linolenic acid (18:3*n*-3) tend to have the lowest *n*6:*n*3 ratios. However the 18:3*n*-3 content of grass decreases with advancing maturity and exposure to shading (reviewed by Dewhurst *et al.*, 2006). With a large proportion of UK lambs finished during late autumn when pasture may have a low nutritional value, the health benefits of late season lamb could be reduced. The aims of the current trial were to investigate the effects of lamb finishing system and fish oil supplementation on the fatty acid profile of sheep meat, produced during autumn and winter finishing.

Materials and methods Eighty entire male lambs (35±5.8 kg) were allocated after weaning to one of five finishing treatments (n=16), balanced for genotype and live weight. Lambs were sourced from six hill farms and represented the offspring of five ewe genotypes (Scottish Blackface, BF; Cheviot X BF; Swaledale X BF; Lleyen X BF, Texel X BF) crossed with Texel, Lleyen and Dorset rams. From September to January, lambs either grazed mixed perennial/Italian ryegrass swards or were housed on concentrate-based diets. Two iso-nitrogenous concentrates (167 g crude protein/kg dry matter) were offered a standard barley-based concentrate or standard concentrate enriched with 35 g/kg herring/mackerel oil. The treatments were as follows: grass-only (control); grass plus 400 g/d standard concentrate; grass plus 400 g/d fish oil enriched concentrate; ad lib standard concentrate indoors and ad lib fish oil concentrate indoors. Housed lambs were also offered 50 g/d chopped straw. After slaughter at 42 or 50 kg, a loin chop (*Longissimus dorsi*) was removed for dissection into adipose and muscle tissues. Lipid was extracted using chloroform:methanol and fatty acid methyl ester analysis was carried out on these tissues using capillary column gas-liquid chromatography. Data were analysed using regression analysis. Contrasts were carried out in a 1+ 2*2 factorial arrangement (control + finishing system*concentrate type) with covariates included for ewe genotype, ram genotype, farm of origin and carcass weight. Means were adjusted to a 20 kg carcass weight.

Results There were no significant interactions so only main effects are presented. Meat from intensively-fed housed lambs had higher (P<0.05) levels of 18:2*n*-6 and 22:6*n*-3 and higher total *n*-6 (P<0.01) and total PUFA (P<0.05) contents than meat from pasture-fed lambs. Concentrate supplementation *per se* had no significant effect on any of the major PUFAs in grass-fed lambs. For both intensive and grass-based finishing, enrichment of concentrates with fish oil resulted in greater levels of 22:6*n*-3 (P<0.01) in muscle, as well as higher total *n*-6, total *n*-3 and total PUFA contents (P<0.05). The ratio of *n*-6:*n*-3 fatty acids in muscle tissue were low for all treatments and were not significantly affected by diet.

Table 1 Effect of finishing system and fish oil on the fatty acid composition (mg fatty acid/g tissue) of lamb muscle

Fatty acid	Finishing system				Concentrate-type				
	Grass	Grass + conc	Ad lib conc	s.e.d	Sig	Standard	Fish oil	s.e.d	Sig
18:2 <i>n</i> -6	0.80 ^a	1.34 ^a	2.32 ^b	0.395	*	1.47	2.19	0.256	P < 0.1
20:4 <i>n</i> -6	0.16	0.15	0.07	0.066	NS	0.10	0.13	0.043	NS
Total <i>n</i> -6	1.02 ^a	1.60 ^a	2.66 ^b	0.365	**	1.64	2.62	0.237	*
20:5 <i>n</i> -3	0.13	0.14	0.20	0.076	NS	0.10	0.23	0.049	P < 0.1
22:6 <i>n</i> -3	0.03 ^a	0.06 ^a	0.18 ^b	0.048	*	0.04	0.21	0.031	**
Total <i>n</i> -3	0.97	1.12	1.29	0.166	NS	0.97	1.45	0.108	*
<i>n</i> -6: <i>n</i> -3 ratio	1.66	1.90	2.07	1.001	NS	1.82	2.15	0.649	NS
Total SFA	33.8	35.7	36.7	6.57	NS	37.4	35.0	4.26	NS
Total PUFA	3.18 ^a	3.90 ^{ab}	4.57 ^b	0.392	*	3.70	4.77	0.254	*

SFA, saturated fatty acids; PUFA, polyunsaturated fatty acids; Means sharing the same superscript are not statistically significant (P>0.05)

Conclusion Meat from lambs finished off autumn/winter pasture can be marketed as a healthy product on the basis of its very low *n*-6 content, although the total PUFA content of grass-fed lamb is also lower than that of lambs finished intensively. Concentrate supplementation at pasture, at the low levels typically seen on commercial UK farms, offers some potential to increase the PUFA content of lamb meat but only when enriched with a PUFA source such as fish oil. The *n*-6:*n*-3 ratios were very low for all the dietary treatments, which could be a reflection of the lamb genotypes and specific tissue studied.

Acknowledgement Funding for this project from the Department of Agriculture and Rural Development and AgriSearch is gratefully acknowledged.

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Can the fatty acid composition of grass-fed sheep be maintained in a creep feed system?

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Introduction It is the practise for some farmers to supplement new season lambs with concentrates, often as a creep feed, in order to get them to market early before the price declines. This concentrate is usually based on cereals and would be high in n-6 fatty acids, diluting the beneficial effect of grass grazing which promotes the n-3 polyunsaturated fatty acid (PUFA) content in the meat (Fisher *et al.*, 2000). The purpose of this trial was to assess the addition of linseed to the concentrate, fed in a creep-feed system, which would maintain or enhance the n-3 fatty acid composition of the meat of animals fed concentrates at grass during the finishing period (Cooper *et al.*, 2004).

Materials and methods Sheep were gathered from the farmer participants to produce a flock of 80 sheep which were divided into four balanced groups of 20 each. One group was finished indoors on a total concentrate diet (Lambmaster, Wynnstay Plc, main components wheat, barley, wheat feed, rapeseed extract and palm kernel expellers) with no green forage (Concs). The remaining groups of 20 were finished in one field which had been divided into paddocks. One group was finished on grazed grass alone (GrGr) while the others also had access to one of two concentrate diets (Lambmaster, GrConcs; Lambmaster with 10% whole linseed replacing some of the wheat and rapeseed, GrLin). After six weeks the lambs were slaughtered at a local abattoir, loin muscle being sampled from 12 animals in each group. Frozen sub-samples were analysed for fatty acid composition and vitamin E content. Loin steaks, conditioned for 10 days, were cut, packed in modified atmosphere packs (O₂:CO₂, 80:20) and subject to simulated retail display (16h light, 8h dark, 3°C, 700lux). Colour on the surface of the steaks was measured through the pack lid daily with a Minolta-Chromameter (Minolta Camera Company, Milton Keynes). Steaks in further packs were taken on day 7 of display and analysed for lipid oxidation as thiobarbituric-acid reacting substances (TBARS). An ANOVA with diet as the main factor was used. And means compared by least significant difference.

Results Feeding concentrates improved the carcass weight and conformation at slaughter over the grass grazed group and reduced the muscle content of 18:3n-3 and increased 18:2n-6. Incorporating linseed in the feed retained the additional 18:2n-6 whilst increasing 18:3n-3, above that of the grass grazed animals, resulting in the GrLin group having the highest content of PUFAs, giving this group the best P:S ratio whilst retaining a good 18:2n-6/18:3n-3 ratio. Vitamin E decreased as the amount of grass in the diet decreased, having no effect on colour stability but increasing the susceptibility to lipid oxidation (TBARS), which was at an unacceptable level in the concentrate-fed group.

Table 1 Diets effects on carcass composition, fatty acids and meat stability

		Concs	GrConcs	GrGr	GrLin	sed	sig
Cold carcass weight	kg	22.1 ^b	22.5 ^b	19.9 ^a	22.7 ^b	0.89	*
Fatness	Scale 4-20	13.3	12.8	12.8	13.5	0.52	ns
Conformation	Scale 1-5	3.5 ^{ab}	3.5 ^{ab}	3.2 ^a	3.8 ^b	0.19	*
% composition total FA							
stearic	18:0	14.04 ^a	14.52 ^a	17.47 ^b	15.13 ^a	0.693	***
oleic	9cis18:1	35.27 ^b	33.86 ^b	34.16 ^b	32.21 ^a	0.698	***
linoleic	18:2n-6	4.48 ^b	3.71 ^b	2.79 ^a	3.85 ^b	0.432	**
alpha-linolenic	18:3n-3	0.88 ^a	1.15 ^a	1.48 ^b	2.07 ^c	0.152	***
CLA	CLA9c11t	0.78 ^a	1.09 ^b	1.18 ^b	0.92 ^{ab}	0.124	*
EPA	20:5n-3	0.46 ^a	0.58 ^{ab}	0.66 ^b	0.73 ^b	0.090	*
DHA	22:6n-3	0.15	0.21	0.19	0.22	0.030	ns
P:S ratio		0.14 ^b	0.13 ^{ab}	0.10 ^a	0.16 ^b	0.015	**
18:2n-6/18:3n-3		6.19 ^b	3.48 ^a	1.93 ^a	1.86 ^a	0.552	***
TBARS	g/kg	2.82 ^c	1.89 ^{bc}	0.55 ^a	1.66 ^{ab}	0.612	**
Vitamin E	g/kg	1.87 ^a	2.91 ^b	4.60 ^c	2.94 ^b	0.286	***
Colour chroma d10		14.6	14.9	14.2	14.7	1.04	ns

Conclusions Providing a creep feed for lambs finished at grass improves carcass growth and conformation. However, it is necessary to provide a good source of 18:3n-3, such as linseed, in order to retain the beneficial fatty acid composition of grass-grazed animals. Supplementing these diets with vitamin E would help maintain the lipid stability of the meat as there is lower vitamin E concentration in concentrate based feeds compared to grass.

Acknowledgements The authors gratefully acknowledge funding from HCC, support of The CardiFarmers consortium and Wynnstay Plc who supplied the creep feeds.

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Improvement of the n-3 fatty acids content in meat of Belgian Blue culled cows and growing fattening bulls offered a diet supplemented with linseeds

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Introduction It is recommended that the n-3 fatty acids intakes and the polyunsaturated/saturated fatty acids ratio be increased and the n-6/n-3 ratio reduced below 5 to reduce the incidence of cardiovascular diseases and cancers in humans. Linseeds are commonly used to increase the n-3 content in meat as they are an important source of C18:3 n-3 fatty acids. Owing to their small size, linseeds must be processed before being offered to cattle. The aim of this study was to compare the inclusion of crushed, flaked and extruded linseeds in a fattening diet of Belgian Blue cattle.

Materials and methods Thirty-two culled cows and twenty growing fattening bulls were divided in four groups. They were offered *ad libitum* a concentrate fattening diet based on sugar beet pulp along with straw. There was no linseed in the control group. Linseed either crushed, flaked or extruded was included at a rate of 65g/kg in the concentrate for the three other groups. On the end of the experiment, three muscles: *Longissimus thoracis*, *Semitendinosus* and *Rectus abdominis* were sampled three days after slaughter when the carcass was cut at the meat plant and analysed for fat and fatty acids contents.

Results The dietary fat content was 25g/kg in the control concentrates and 50g/kg in the concentrates with linseeds. The corresponding C18:3 n-3 contents were 3.9 and 12.8 g/kg. The inclusion of linseeds did not affect the fat content in meat (Table 1). The meat of the growing fattening bulls was leaner than that of the culled cows (13.1 vs 34.2 g fat/kg dry matter - DM). The C18:3 n-3 content was quite similar in the meat of both control culled cows and bulls. The inclusion of linseed improved by about 50% the content of C18:3 n-3 ($P < 0.001$) with no differences between linseeds treatments. The content of total n-3 fatty acids was also improved by the inclusion of linseeds but the difference was significant only for the meat of culled cows. However, there were no effects of the inclusion of linseeds on the polyunsaturated fatty acids (PUFA) contents in meat which remained in the same range for the cows and the bulls as indicated in figure 1 in which the PUFA and the sum of the saturated (SFA) and the monounsaturated fatty acids (MUFA) were expressed according to the fat content. PUFA content remained quite constant as animals fattened whilst SFA+MUFA increased. There was a significant decrease of the n-6/n-3 ratio when linseeds were included but still with no differences between treatments for culled cows.

Table 1 Fat and fatty acids concentrations in meat of culled cows and of growing fattening bulls offered a control fattening diet or a diet in which linseeds were either crushed, flaked or extruded.

	Culled cows				Growing fattening bulls				SEM	P<F		
	Con. ⁽¹⁾	Cru. ⁽²⁾	Fla. ⁽³⁾	Ext. ⁽⁴⁾	Con. ⁽¹⁾	Cru. ⁽²⁾	Fla. ⁽³⁾	Ext. ⁽⁴⁾		Diet	Sex	Muscle
Fat ⁽⁵⁾	31.2	35.7	37.3	32.7	13.4	11.9	16.3	11.0	0.18	NS	***	**
Fatty acids												
C18:3 n-3 ⁽⁶⁾	14.3 ^a	21.5 ^b	21.4 ^b	21.1 ^b	13.4 ^a	20.2 ^b	18.2 ^b	18.8 ^b	0.50	***	*	NS
Sum ⁽⁶⁾	862	938	1091	891	469	405	457	393	45.24	NS	***	**
Σ n-6 ⁽⁶⁾	130.0 ^a	123.0 ^a	119.0 ^a	118.2 ^a	165.4 ^a	141.8 ^b	152.5 ^{ab}	153.5 ^{ab}	1.95	*	***	*
Σ n-3 ⁽⁶⁾	42.3 ^a	52.2 ^b	51.5 ^b	52.2 ^b	31.7	37.8	35.8	35.8	0.94	**	***	***
n-6/n-3	3.1 ^a	2.3 ^b	2.3 ^b	2.2 ^b	5.3 ^a	3.7 ^b	4.2 ^c	4.3 ^c	0.06	***	***	***

⁽¹⁾control ⁽²⁾crushed linseeds ⁽³⁾flaked linseeds ⁽⁴⁾extruded linseeds ⁽⁵⁾g/kg DM ⁽⁶⁾mg/100g fresh meat

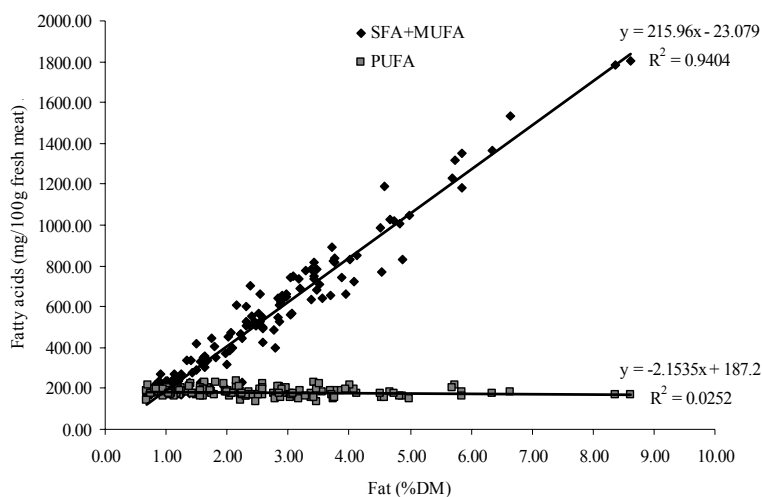


Figure 1 Relationship between the fatty acids and fat contents in meat

Conclusion It was concluded that the use of linseeds significantly increased the C18:3 n-3 content in the meat of both culled cows and growing fattening bulls with no differences between the three treatments.

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Effect of supplementing feed with vitamin D₃ and calcium propionate on lamb meat quality

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Introduction Toughness is a determinant of meat quality, a common cause of unacceptability in meat products and consumers are willing to pay higher prices for meat which is guaranteed to be tender. The calpain proteolytic enzymes are involved in the process of meat tenderisation through their degradation of muscle proteins postmortem (Sensky *et al.*, 2001) and their activity is influenced by fluctuating levels of Ca²⁺, pH and temperature, all of which change rapidly in the immediate postmortem period. As vitamin D (vit D₃) is responsible for Ca²⁺ homeostasis, we hypothesised that supplementing feed with vitamin D or vitamin D plus calcium propionate (CaPr) before slaughter would result in more tender meat through enhanced postmortem calpain system activity.

Materials and methods Wether lambs were allocated to two treatment groups consisting of paired control (n=18) and vit D₃ treatments (n=18). All animals were individually housed and fed 1.4 kg/day of diet containing (fresh weight basis) 480 g/kg barley, 160 g/kg oats, 200 g/kg grass meal and 100 g/kg Nutramol 30 (Rumenco, UK) with no added vitamins or mineral supplements. Trial 1: Treatment group's diet was supplemented with ROVOMIX D3 500 (DSM, UK) at 2 x 10⁶ IU Vit D/day for 4 days before slaughter. Trial 2: Treatment group's diet was supplemented with vit D₃ at 2 x 10⁶ IU Vit D/day for 7 days before slaughter and on the day before slaughter with CaPr to increase feed Ca from 7.0 g/kg Dry Matter (DM) to 14.0 g/kg DM. For both trials blood samples were taken at slaughter for the determination of serum Vit D₃ levels (Trial 1, by tandem mass spectrometry; Trial 2, by HPLC) and plasma Ca²⁺ by atomic absorption spectrometry. Carcasses were stored at 4°C for 24 hours then the *longissimus dorsi* (LD) was dissected, vacuum packed and aged for 7 or 14 days at 4°C prior to Warner Bratzler Shear Force (WBSF) analysis. Data were analysed using paired *t*-test for the blood parameters or two-way ANOVA (treatment x time) for WBSF.

Results In both trials there were significant increases in the levels of serum Vit D₃ in response to supplementation; whilst CaPr supplementation significantly increased plasma Ca²⁺ concentrations in Trial 2 (Table 1). In Trial 1 there was no effect of vit D₃ treatment on LD WBSF, but there was a significant decrease in WBSF between the two time points (P<0.05, Figure 1A). In Trial 2 there was a trend for vit D₃ + CaPr treated lambs to have higher WBSF (P=0.06) and there was a significant increase between the two time points (P<0.01, Figure 1B).

Table 1 The effect of supplementing feed with Vit D₃ (Trial 1) or Vit D₃ and CaPr (Trial 2) on serum Vit D₃, plasma Ca²⁺

	Trial 1				Trial 2			
	Control	Vit D ₃	SEM	P	Control	Vit D ₃	SEM	P
Serum Vit D ₃ , ng/ml	28.0 ^a	319.6 ^a	19.2	<0.001	49.9	1230.0	54.32	<0.001
Plasma Ca ²⁺ , mg/100ml	11.9	12.2	0.49	NS	12.9	14.3	0.33	<0.001

^a n=17

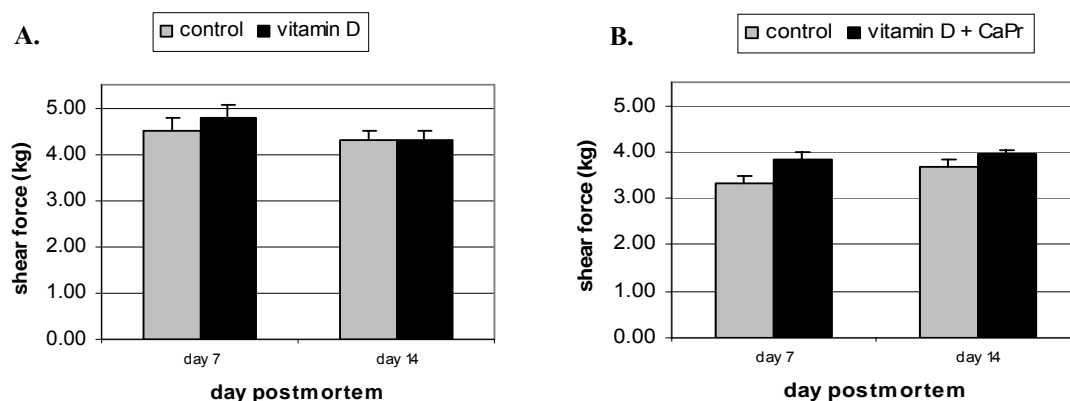


Figure 1 The effect on LD WBSF of supplementing feed with A. Vit D₃ (Trial 1) or B. Vit D₃ and CaPr (Trial 2)

Conclusion Although previous studies have found that feeding cattle with elevated levels of vit D₃ for a week or longer prior to slaughter enhanced meat quality (Montgomery *et al.*; 2004), in lambs we failed to observe any reduction in shear force (meat toughness) although serum vit D₃ and plasma Ca²⁺ concentrations were increased.

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Simulated Global Warming Potential figures for UK suckler beef production systems

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Introduction In line with international treaties, UK Government policy seeks to diminish the Global Warming Potential (GWP) of agricultural activities through reductions in Greenhouse Gas (GHG) emissions associated with livestock production. For suckler beef production systems these GHGs are principally methane (CH₄) and nitrous oxide (N₂O) associated with rumen fermentation of feeds consumed and management of manure produced by animals and spread on land. The objective of this simulation exercise was to simulate the GHG emissions and therefore GWP associated with a range of suckler beef production systems potentially available to current and future UK beef producers.

Materials and methods The suckler beef production systems simulated were based on five potential suckler herd management strategies (HS) as follows:- dairy x beef suckler cows (Dx), pure bred beef cows (PB), a 3-way rotational beef breed crossing strategy (RO), a “composite” beef herd breeding strategy (CO) and a future beef herd management strategy based on the availability and use of sexed semen (SS). For the PB, RO, CO and SS strategies replacement heifers were assumed to be home bred whilst for the Dx strategy replacement heifers were assumed to be purchased as calves and reared within the system. In addition to these 5 herd management strategies, simulations were also conducted according to either a 12, 18, 24 or 30 month weaned calf finishing system (FS) giving a total of 20 system combinations for comparison. Duplicate simulations were carried out for each of these 20 combinations by assuming two typical, yet contrasting breed types (based on UK & continental breeds) within each of the 20 possibilities. Both CH₄ and N₂O outputs were simulated on the basis of the Tier 2 methods adopted by the Intergovernmental Panel on Climate Change (IPCC, 1996, 2000) whilst cattle data parameters were derived using the BREEDS bioeconomic model for the various system combinations assumed (Roughsedge *et al.*, 2003). Both CH₄ and N₂O simulated figures were converted from GHG outputs into GWP figures for each of the duplicate system combinations according to the conversion factors assumed by IPCC 1996 and expressed as tonnes of CO₂ equivalents per year. Figures were generated for an assumed suckler herd of 100 cows, plus all associated heifer replacements along with all weaned calves through to slaughter. All simulated GHG emission and GWP figures were statistically analysed by ANOVA using Genstat 5 according to a 5 x 4 (HS x FS) factorial experimental design.

Results Total GWP figures for each 100-cow system, for each of the 20 HSxFS combinations are given in Table 1. Both HS (P<0.05) and particularly FS (P<0.001) significantly influenced the GWP of the simulated 100 cow suckler herd system. The FS in particular, progressively increasing GWP from 316, 365, 397 to 462, t/year for the 12, 18, 24 and 30 months FS respectively with the largest effect being seen within the Dx and SS HS. The relative GWP contributions from the suckler herd, heifer replacements and finishing cattle sections of the overall simulated 100-cow herds are shown in Figure 1.

Table 1 Simulated GWP output figures for different HS x FS suckler beef production systems (t CO₂ eq / 100-cow system)

(HS) Herd	Finishing system (FS)				Mean	Sig of effects		
	12	18	24	30		HS	FS	HSxFS
Dx	360	410	450	534	438 ^a			
PB	301	343	371	429	361 ^b			
RO	316	361	390	450	380 ^c			
CO	306	352	381	447	372 ^d			
SS	295	358	392	449	373 ^d			

Mean 316^a 365^b 397^c 462^d * *** *

Sed's: HS 1.7; FS 1.5; HSxFS 3.3.

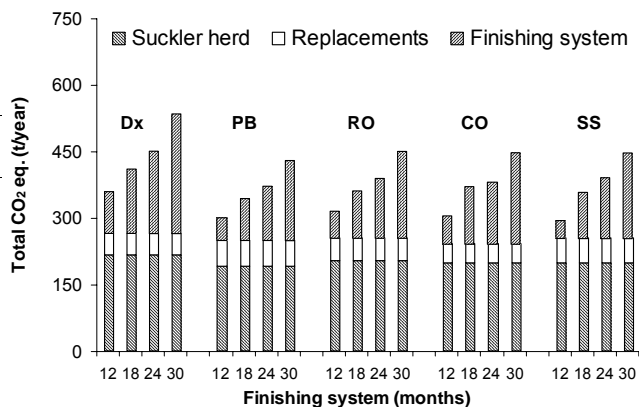


Figure 1 Relative GWP contributions from the suckler breeding herd, heifer replacements and finishing cattle

Conclusions Simulations suggest that some opportunities exist to reduce GHG emissions through optimum choice of suckler herd management strategy. In particular, these simulations also suggest that intensive, feed efficient finishing systems that slaughter animals at younger ages could substantially reduce the GWP of UK suckler beef production systems.

Acknowledgements Defra provided the funding for this simulation analysis.

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Simulated ammonia emission figures from UK suckler beef production systems

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Introduction In line with international treaty obligations, UK Government policy seeks to reduce ammonia (NH₃) emissions from livestock production systems. For suckler beef production systems NH₃ release is principally associated with cattle grazing, emissions during winter housing, storage and management of animal manures and spreading of both animal manures and artificial fertilisers on land. The objective of this simulation exercise was to simulate the NH₃ emissions associated with a range of suckler beef production systems potentially available to current and future UK beef producers.

Materials and methods The suckler beef production systems simulated were based on five potential suckler herd management strategies (HS) as follows:- dairy x beef suckler cows (Dx), pure bred beef cows (PB), a 3-way rotational beef breed crossing strategy (RO), a “composite” beef herd breeding strategy (CO) and a future beef herd management strategy based on the availability and use of sexed semen (SS). For the PB, RO, CO and SS strategies replacement heifers were assumed to be home bred whilst for the Dx strategy replacement heifers were assumed to be purchased as calves and reared within the system. In addition to these 5 herd management strategies, simulations were also conducted according to either a 12, 18, 24 or 30 month weaned calf finishing system (FS) giving a total of 20 system combinations for comparison. Duplicate simulations were carried out for each of these 20 combinations by assuming two contrasting breed types within each of the 20 possibilities. NH₃ outputs were simulated on the basis of the emissions factors detailed by Misselbrook *et al*, (2000) and Misselbrook, (2003) whilst cattle data parameters were derived using the BREEDS bioeconomic model for the various system combinations Roughsedge *et al*, (2003). Total NH₃ outputs were calculated for all of the major sources listed above and expressed as kg of NH₃ per year. Figures were generated for an assumed autumn calving suckler herd of 100 cows, plus all associated heifer replacements along with all weaned calves through to slaughter. All simulated NH₃ emission figures were statistically analysed by ANOVA using Genstat 5 according to a 5 x 4 (HS x FS) factorial experimental design.

Results Total NH₃ emission figures for each 100-cow system, for each of the 20 HSxFS combinations are given in Table 1. Both HS (P<0.05) and particularly FS (P<0.001) significantly influenced the NH₃ emissions of the simulated 100 cow suckler herd system. The 30 month FS in particular, increasing NH₃ emissions to 2388 kg/year from 1773, 1875, 1881 for the 12, 18 and 24 months FS respectively. The relative NH₃ emission contributions from the suckler herd, heifer replacements and finishing cattle sections of the overall simulated

Table 1 Simulated NH₃ output figures for different HS x FS suckler beef production systems (kg / year / 100-cow system)

(HS)	Finishing system (FS)				Mean	Sig of effects		
	12	18	24	30		HS	FS	HSxFS
Dx	1929	2026	2038	2647	2160 ^a			
PB	1784	1873	1879	2381	1979 ^b			
RO	1757	1854	1860	2332	1951 ^{bc}			
CO	1713	1807	1810	2294	1906 ^c			
SS	1680	1815	1819	2288	1900 ^c			

Mean 1773^a 1875^b 1881^b 2388^c * ** *

Seds: HS 28.9; FS 25.9; HSxFS 57.9.

Values not sharing common superscripts differ significantly.

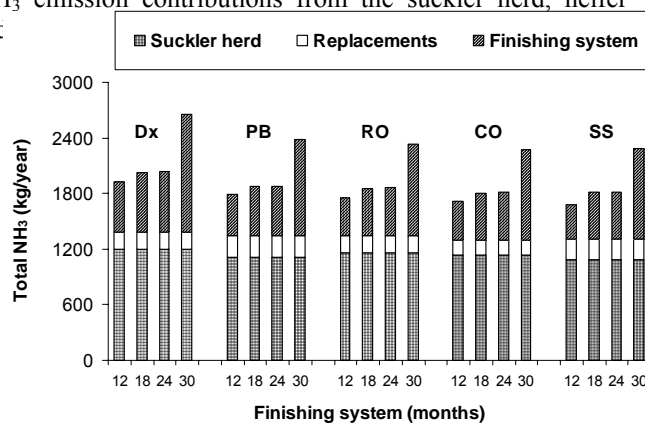


Figure 1 Relative NH₃ contributions from the suckler breeding herd, heifer replacements and finishing cattle

Conclusions Simulations suggest that some limited opportunities exist to reduce NH₃ emissions through optimum choice of suckler herd management strategy. However, these simulations also suggest that the greatest opportunity to reduce NH₃ emissions from UK suckler beef production systems can be found in the adoption of intensive, feed efficient finishing systems that slaughter animals at younger ages, particularly in reducing slaughter ages from 30 to less than 24 months.

Acknowledgements Defra provided the funding for this simulation analysis.

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Effect of dietary inclusion of encapsulated fumaric acid on methane production from grazing dairy cows

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Introduction Organic acids, most commonly fumarate and malate, when offered to ruminants have been shown to produce a decrease in methane emissions along with an increase in *in vitro* DM digestibility in rumen fluid taken from sheep (López *et al.*, 1999). They are classed as 'preservatives' in the list of feed additives authorised by EU legislation, and as such are permitted for use in livestock (Castillo *et al.*, 2004). However, the addition of fumaric acid to the diet has been associated with inappetence and problems of reduced rumen pH. Fumaric acid encapsulated in a soya oil capsule enables a slow release of fumaric acid in the rumen, which should overcome these problems. Encapsulated fumaric acid (EFA), when offered to lambs as 10% of the diet, reduced methane emissions by 75% and increased feed conversion efficiency by 20% (Wallace *et al.*, 2006). To date, no *in vivo* studies have been carried out on dairy cattle. Therefore, the objective of this study was to determine the effect of addition of EFA on animal performance and methane production from grazing dairy cows.

Materials and methods Twelve first lactation Holstein dairy cows were used in a repeated Latin Square design study (3 treatments x 3 periods, with 3 weeks/period) to investigate the effect of EFA on methane emissions. The EFA was offered at three levels of inclusion (0, 0.7 and 1.4 kg/d), and mixed with 5 kg fresh concentrate per day. The EFA consisted of fumaric acid (87%) and soyabean oil (13%). To balance the effects of the soyabean oil and fumaric acid on the rumen environment, glucose and soyabean oil were added to the control and low EFA treatments to the same levels of fumaric acid and soyabean oil in the high EFA treatment. The cattle were grazing for the duration of the study and offered their treatment diet split equally morning and evening at milking time. Individual grass intake was measured using the *n*-alkane technique during the final week of each period, and rumen samples were taken four times throughout the duration of the study (prior to commencement of the study and on the final day of each of the three periods) to determine any change in rumen fermentation parameters and microbial population. Methane production was measured using the sulphur hexafluoride (SF₆) tracer technique for the final four days of each period. The data were analysed using a one-way ANOVA with animals as randomised blocks.

Results Animal performance and methane output for the three treatments is presented in Table 1. The addition of EFA had no significant effect on liveweight, total DM intake, milk yield or composition or rumen fermentation parameters. The high EFA treatment resulted in significantly reduced numbers of *Ruminococcus flavefaciens* ($P < 0.05$). Numbers of ciliates, anaerobic fungi, archaea, *Butyrivibrio* and *Fibrobacter succinogenes* were unaffected by treatment. There was no significant effect of EFA on methane production, either expressed as L/day, or as a proportion of milk yield, total DM intake, liveweight or total ME intake.

Table 1 Methane output for the three treatments.

	Control	Low EFA	High EFA	S.E.	Sig ¹ .
Liveweight (kg)	466	469	466	6.1	NS
DM intake (kg/d)	10.65	10.38	10.77	0.963	NS
Milk yield (kg/d)	14.3	13.8	14.5	0.48	NS
Mean CH ₄ (L/d)	389	386	390	44.5	NS
CH ₄ / DM intake (L/kg)	36.5	37.2	36.2	4.45	NS
CH ₄ /milk yield (L/kg)	27.2	28.0	26.9	3.48	NS
CH ₄ /liveweight (L/kg)	0.78	0.75	0.82	0.13	NS
CH ₄ / total ME intake (L/MJ)	3.04	3.22	2.70	0.381	NS

1. NS = not significant

Conclusions The addition of encapsulated fumaric acid, in the present formulation, to the concentrate rations of grazing dairy heifers produced no significant effect on rumen fermentation, methane output or performance parameters (such as liveweight, DM intake and milk yield and composition). These results indicate that this formulation of encapsulated fumaric acid cannot be used as a mitigation strategy to reduce methane production from grazing dairy cows.

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The effect of different forage based diets on enteric methane emissions from dairy cows

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Introduction Methane from enteric fermentation is a large component of livestock related greenhouse gas emissions. Typically, methane emissions from enteric fermentation represent on average 6% of dietary gross energy, but this varies greatly with diet (Johnson and Johnson, 1995). In order to mitigate methane emissions in a way that is acceptable for both the environment and animal welfare, it is important to quantify the effects of different diets on methane emissions. The objective of the current study was to determine the effect of different silages on methane emissions from dairy cows.

Materials and methods Fifty individual silages samples produced and utilised at the SAC's Crichton Royal Farm, Dumfries, from 2002 to 2007 were studied for their potential production of methane. The silages were from first to third cut within each year. The analytical composition of the silages indicated an average dry matter content of 247.8 (sd = 66.7) g/kg, 146 (sd = 26.2) g/kg DM crude protein, 104 (sd = 80.9) gN/kgTN ammonia, 502.6 (32.5) g/kg DM NDF, and 11.1 (0.6) MJ/kg DM ME. In the analysis, the calculations were based on a scenario where the different silages were fed to a standard high milk producing cow. The cow was described to be in second parity and as having the live weight of 700kg and a body condition score of 2.5 (0 to 5 scale) (Mulvanny, 1977). The cow was assumed to be losing 0.5 live weight kg per day and being in the 12th week in lactation. The cow had the potential milk yield of 30kg per day with the milk composition of 39g/kg fat and 34g/kg protein. The data for each ration were run in FeedByte, SAC's nutrition software based on least cost diet formulation and linear programming modelling (SAC, 1999). The runs from FeedByte among other outputs produced dry matter intake for the cow and the potential amount of milk the cow would produce per day from silage alone. Enteric methane emission per cow was estimated using two different equations. Equation one (methane1) estimated methane mass produced as a function of the animal gross energy intake per day (Casey and Holden, 2006). The second equation (methane2) accounted for the role of maintenance energy requirements and the role of digestibility in methane emissions (Blaxter and Clapperton, 1965). Data were analysed using ANOVA with year and silage cut as fixed effects.

Result The general trend for milk yield and CH₄/l milk are presented in Figure 1. The silages which sustained low milk yield had higher CH₄/l milk that those silages that sustained high milk yield. On average the cows produced 22 (sd =5.57) kg of milk per day. Per litre of milk, the cows emitted on average 0.013 (sd = 0.004) kg of methane. The year in which the silages were produced had a significant effect on methane emissions (p<0.001) (Table 1). The silage cut had no significant effect on methane emissions.

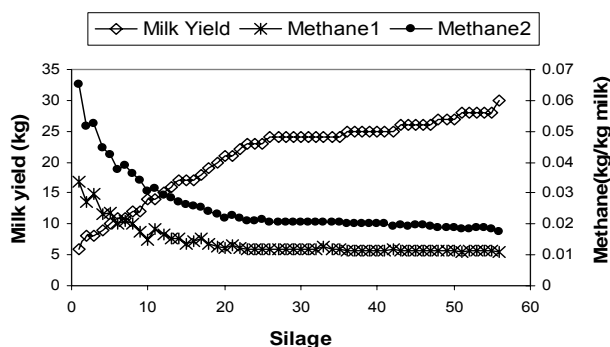


Figure 1 Trends for milk yield and methane per kg of milk for the different silages

Table 1 Year and silage cut effects on methane emissions

Factor	Methane1		Methane2	
	Lsmean (kg/kg milk)	SEM	Lsmean (kg/kg milk)	SEM
Year				
2002	0.018 ^a	0.0009	0.033 ^d	0.0027
2003	0.012 ^b	0.0007	0.020 ^c	0.0031
2004	0.011 ^b	0.0008	0.020 ^c	0.0035
2005	0.012 ^b	0.0019	0.022 ^e	0.0080
2006	0.012 ^b	0.0012	0.022 ^e	0.0012
2007	0.013 ^b	0.0011	0.023 ^e	0.0017
Cut				
1	0.014 ^c	0.0006	0.024 ^f	0.0018
2	0.012 ^c	0.0008	0.022 ^f	0.0002
3	0.013 ^c	0.0012	0.024 ^f	0.0016

Different superscripts within factor for each methane estimate indicate significant difference (p<0.001)

Conclusions The results indicate that the between-year variation in the silage quality affected methane emission from the diets for dairy cows more than the within-year variation emanating from different silage cuts. Silage quality affects the potential methane emissions of any particular silage.

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Opportunities for genome wide selection within New Zealand livestock industries

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Introduction Livestock selection programmes have progressed from visual assessments to the use of quantitative information from multiple sources. More recently genetic markers have been used to incorporate information from quantitative trait loci (QTL) into selection decisions. The uptake this technology has been slow due to the cost and difficulty of discovering and using QTL. For a number of livestock species single nucleotide polymorphism (SNP)-chips are becoming available. These have the ability to genotype 10,000s of SNP markers in a single assay. This opens up the possibility of 'genome wide selection' (GWS) to 'tag' most of the genetic variants contributing to trait differences. This paper discusses what impact GWS might have in the main NZ livestock breeding industries (dairy, beef, sheep and deer).

GWS characteristics GWS requires a dense marker set to capture most of the information associated with each genetic variant affecting traits of interest. An estimation step is required to quantify the marker effects on these traits in a relevant set (the 'training set') of animals, preferably using earlier generations of the population (if recorded for the relevant phenotypes). The training set should avoid confounding of environments and genotypes and match the breed of application. Appropriate statistical methods are required to make predictions with such high-dimensional data (Meuwissen *et al.* 2001). GWS can be applied without pedigree information, as it endeavours to capture all the genetic information with markers. It can be applied to unphenotyped animals, allowing individuals to be selected at a younger age than previously practiced. Theoretical studies (Meuwissen *et al.* 2001) indicate that the accuracy of selection with GWS is high (on a par with progeny testing). Practical situations may result in some reduced accuracy (Hayes & Goddard 2007).

GWS in dairy cattle The NZ dairy industry has wide-spread use of a few highly selected sires following progeny testing. GWS would allow the generation interval to be decreased by by-passing progeny testing. Schaeffer (2006) estimates that GWS in Canadian Holsteins would cost less than 10%, and more than double the genetic gain per year, compared to the present situation. In the NZ dairy industry the costs would probably be around 50% of the current system. A 60,000 marker SNP-chip has been developed for cattle, which should be sufficient to capture a large proportion of the genetic information (Hayes *et al.* 2006). Stored DNA samples of many of the sires used since the early 1980's could be used in a training set. The two largest AI companies have started using GWS to select among candidate sires and reduce the number undergoing progeny testing. This allows the methodology to be demonstrated before commercial farmers are presented with GWS-, non progeny tested – selected bulls. Additional gains from using GWS could be made by incorporating information on traits such as longevity and fertility that are not normally available until after selection of bulls for widespread usage.

GWS in sheep, beef and deer GWS is less attractive and less imminent for these industries than in the dairy industry. They generally use little progeny testing, dissemination of genetics is through multipliers (rather than AI), there are several breeds and animals have lower value. Selection is mainly on traits (such as liveweight) that are available before breeding. The main advantage of GWS in these industries would be for traits such as meat quality, fertility (including calving date for deer) and health. Training sets could be developed by progeny testing industry sires or by measuring the traits in sets of animals representative of industry. It will be advantageous to measure as many phenotypes as practical on the same sets of animals.

The application of GWS to maternal traits, such as fertility and survival, is likely to result in the development of separate maternal and paternal breeds or lines for beef and deer (already exist for sheep). For GWS to be feasible there needs to be a pathway to recover the costs. An AI company would need to be confident of an upsurge in AI before embarking on GWS to select sires. The value of the additional gains possible by using GWS-selected sires needs to outweigh direct and indirect (e.g. management) costs of using AI for the producer. Alternatively GWS could be undertaken by cooperatives, corporate farms or industry bodies. Recovery of costs for meat quality traits would require vertically integrated structures and product branding to avoid costs of measuring individuals at slaughter.

The beef industry can use the cattle SNP-chips already developed. A SNP discovery project is currently in progress for sheep, to allow a suitable SNP-chip to be developed. The deer industry is relatively small and SNP-chip development is likely to be delayed until its cost drops further. GWS is likely to be initially applied at the nucleus tier (sires to breed sires). If early results show that SNP subsets can be used with reasonable accuracy, and platforms are available for relatively cheap genotyping of these subsets, then GWS may eventually be applied at the multiplier, or even producer, level. Producers may choose to become their own multipliers, as pedigrees and phenotypes of candidates are no longer required.

Conclusion GWS could have a major impact on the New Zealand livestock industries. It is currently being trialled in the dairy industry which has sufficient marker resources, appropriate training sets, structures that allow a return to the investor, and appears very likely to be economically viable, mainly through reducing generation intervals. The other industries require structures that allow a financial return to entities that invest in GWS. They are likely to apply GWS to non-production traits such as fertility, health and product quality. The industries differ in when suitable SNP-chips are likely to be available. Application of GWS may be delayed until genotyping costs drop sufficiently to catalyse the industry changes needed.

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Genomic scan for quantitative trait loci of pig chemical and physical body composition and growth traits on chromosome X

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Introduction Identification of quantitative trait loci (QTL) provides insight into the genetic control of growth and body composition in pigs. The majority of QTL have been identified on autosomes with less QTL reported on the sex chromosomes. A reason may be that the genomic analysis of the X chromosome is more statistically challenging. Computer programmes accounting for the unique features of chromosome X have been unavailable until recently. Most studies have adopted a regression-based approach analysing males and females separately, which results in a decrease in power to detect QTL. In the present study, a QTL analysis of pig chromosome X (SSCX) was carried out using a method which accounts for the unique features associated with chromosome X, including the pseudoautosomal region of the Y chromosome.

Materials and methods QTL mapping was based on data from a 3 generation full-sib design (Pietrain x commercial dam line). Phenotypic data were available for 315 F2 animals for dissected carcass cuts and carcass measurements obtained from the AutoFOM system measured at slaughter weight (140 kg body weight), chemical body composition of the empty body and protein and lipid deposition of live animals throughout growth from 30-140 kg. Data were also available for daily gain (DG), daily feed intake (DFI) and food conversion ratio (FCR). 386 animals from 49 families were genotyped for 8 informative microsatellite markers on SSCX. QTL mapping was carried out using a maximum likelihood approach with QxPak 2.16 (Perez-Enciso and Misztal, 2004).

Results In the pseudoautosomal region of SSCX at 7 cM, a significant QTL was identified for entire loin weight, accounting for 3.7% of the phenotypic variance. In a similar location, a suggestive QTL was identified for loin lean meat weight (Figure 1). Additive effects indicate that the grandpaternal Pietrain alleles are associated with increased weights of these carcass cuts. A suggestive QTL was identified for DFI 120-140 kg (56 cM) explaining 2.3% of the phenotypic variance. At this QTL, Pietrain alleles are associated with increased feed intake. At the telomeric end of the q arm of SSCX at 128.4 cM, significant QTL were identified for lipid accretion rate (LAR) at 90-120 kg and jowl weight explaining 3.2 and 5.8% of the phenotypic variance, respectively (Figure 2). At these QTL, Pietrain alleles are associated with 26.7 g/day higher LAR and heterozygous animals are associated with higher jowl weight. At 82-83 cM suggestive QTL were identified for protein and lipid content of the empty body and protein content of the fat free substance at 30 kg body weight explaining 3.8, 3.3 and 3.1% of the phenotypic variance, respectively (Figure 2).

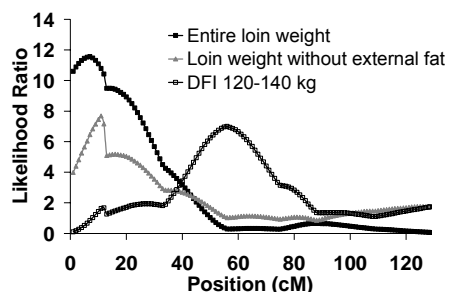


Figure 1 Evidence for QTL for entire loin weight, loin weight without external fat and DFI 120-140 kg

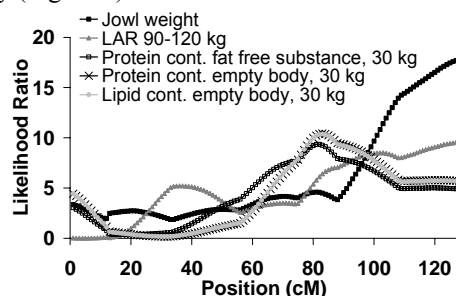


Figure 2 Evidence for QTL for jowl weight, LAR 90-120 kg, and several body composition traits at 30 kg

Conclusion Published evidence of QTL for chemical body composition measured in live animals is limited. In the present study a QTL for LAR was found on SSCX in a region where no QTL for fat tissue was identified (Figure 2). At this QTL, Pietrain alleles are associated with increased LAR. QTL for LAR have been reported in only two previous studies (Mohrmann *et al.*, 2006, Duthie *et al.*, 2007). Surprisingly in the present study, Pietrain alleles were found to be associated with increased feed intake (cryptic) on SSCX. QTL for chemical body composition measured in live animals have only been reported in two previous studies (Mohrmann *et al.*, 2006, Duthie *et al.*, 2007). QTL were identified for chemical body composition at an early stage of growth on SSCX in the present study. Heterozygous animals are associated with increased protein content of the fat free substance and lipid content of the empty body as well as decreased protein content of the empty body. In the pseudoautosomal region of SSCX, Pietrain alleles were found to be associated with increased weights of carcass cuts and lean tissue. In the present study, SSCX has shown important associations with physical and chemical body composition as well as DFI. Information about the genomic regulation of physical and chemical body composition can be used for marker-assisted selection to improve carcass quality and feed efficiency.

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Two *myostatin* single nucleotide polymorphisms have significant effects on muscle depth of British commercial Charollais sheep

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Introduction In 2006, *myostatin* single nucleotide polymorphisms (SNPs) were identified on ovine chromosome 2 (OAR2), with two SNPs (g-2449C-G and g+6723G-A) having significantly higher frequency in hyper-muscled Belgian Texel than in control animals (Clop *et al.*, 2006). In particular, the A allele of the g+6723G-A SNP was proposed to be a causative mutation for increased muscularity in Texel rams (Clop *et al.*, 2006). We aimed to assess whether the above two SNPs were present in UK commercial Texel, Suffolk and Charollais breeds. Moreover, we performed association analysis of the SNP effects on phenotypes in an extended UK commercial population of Charollais sheep in order to further evaluate the SNP contribution to muscle and fat tissue composition, and to quantify and fully characterise the SNP effects in this breed.

Materials and methods Nine Suffolk, 38 Texel, and 34 Charollais rams from UK commercial flocks were first genotyped for the g+6723G-A and g-2449C-G SNPs. Because the Charollais sires were segregating for the two SNPs (see Results), we then obtained SNP genotypic data on 338 Charollais sheep from 17 paternal half-sib families dispersed in 12 commercial flocks. Standard husbandry records and phenotypic data on muscle and fat depth ultrasonically scanned at the third lumbar vertebra and on live weight at scanning were provided by Signet (Meat and Livestock Commission) for 56,500 five-month-old Charollais lambs, including the 338 genotyped animals. Mixed model association analysis of each of the SNP effects on the traits was performed using ASREML (Gilmour *et al.*, 2006). As well as fitting each SNP effect, fixed effects of sex, litter size at birth, litter size reared, year, flock and age of dam were included in each analysis. The age at scanning (in days) was fitted as a covariate. An animal model in which dam and litter were also fitted as random effects was chosen.

Results The A allele of the g+6723G-A SNP, coding for muscular hypertrophy, and the C allele of the g-2449C-G SNP were absent in the genotyped Suffolk sires and almost fixed in the Texel sires. In the Charollais population these alleles were segregating at intermediate frequencies ($p=0.3$ in our sample). Mixed model association analysis revealed that the SNPs had significant effects on the muscle depth of Charollais sheep ($P<0.001$; Table 1). The A allele of the g+6723G-A SNP had an additive effect (a) of 1.20(± 0.30) mm on muscle depth. This is equivalent to the average effect of allelic substitution, assuming equal allele frequencies at a biallelic locus. The dominance effect (d) of the A allele was -0.73(± 0.36) mm (Table 1). The average effect of allelic substitution for the C allele of the g-2449C-G SNP and its dominance effect are also given in Table 1. Assuming equal allele frequencies at a biallelic locus, the g+6723G-A SNP explained 29% of the additive genetic variance (σ^2_A) for muscle depth, whereas the g-2449C-G SNP accounted for 21% of σ^2_A (Table 1). Moreover, pair-wise contrasts of the predicted muscle depth values of each SNP genotype class revealed that animals with the AA genotype at the g+6723G-A SNP position had significantly greater muscle depth than those with either the GG or the AG genotype ($P<0.002$). The animals with the CC genotype at the g-2449C-G SNP also had significantly higher muscle depth than those that had either of the other genotypes at the locus ($P<0.01$). Finally, using the animal/dam/litter model, direct and maternal genetic heritabilities for muscle depth were estimated to be 0.29 (± 0.01) and 0.03 (± 0.005), respectively. The proportion of phenotypic variance due to common environment (litter effect) was estimated as 0.22 (± 0.007). SNP effects on fat depth and live weight were not significant.

Table 1 Animal model SNP association analysis of muscle depth: SNP effects, F-ratios and P-values, allelic effects, and percentage of additive genetic variance (σ^2_A) explained by SNP

Trait	SNP	F-ratio	P-value	Additive effect a (\pm SE)	Dominance effect d (\pm SE)	Percentage σ^2_A due to SNP
Muscle	g+6723G-A	8.05	0.001	1.20(± 0.30)	-0.73(± 0.36)	29
Depth (mm)	g-2449G-C	7.78	0.001	1.00(± 0.25)	-0.45(± 0.33)	21

Conclusions The A allele of the g+6723G-A SNP and the C allele of the g-2449C-G SNP, both in the *myostatin* region of OAR2, segregated in commercial Charollais sheep, and a significant association was found between these polymorphisms and muscle depth. The A allele of the g+6723G-A SNP, which is a proposed QTN for muscularity (Clop *et al.*, 2006), resulted in an increased muscle depth in Charollais sheep and, for intermediate allele frequencies, the SNP explained 29% of the additive genetic variance for the trait. SNP-assisted selection for muscle depth would be beneficial for this breed.

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Effects of a Texel sheep muscling QTL (TM-QTL) on carcass traits in crossbred lambs

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Introduction A QTL (TM-QTL) identified on ovine chromosome 18 (Walling *et al.*, 2004), which increases loin muscle depth by 4-8% in UK Texel sheep, is of interest for the sheep industry as a potential means to increase carcass value. Since the contribution of Texel genes to the UK slaughter generation is generally through use of Texel sires to produce crossbred slaughter lambs (e.g. Texel x Mule lambs), it is necessary to verify the effects of the TM-QTL on loin muscularity and other carcass traits in such crossbred progeny of Texel sires before exploitation of the TM-QTL in commercial sheep populations.

Materials and methods Two-year old Mule ewes (Bluefaced Leicester x Blackface) were mated using AI to four Texel sires heterozygous for TM-QTL. The Texel x Mule lambs produced were classed as either carriers (n=63) or non-carriers (n=48) of a single copy of TM-QTL based on genotypes at four microsatellite markers on ovine chromosome 18. Lambs were reared with their mothers on pasture until slaughter at 21-22 weeks of age. Prior to slaughter, lambs were scanned using ultrasound (average age=141 days), to determine ultrasonic muscle (UMD) and fat depths (UFD) in the loin region, and X-ray computed tomography (CT) (average age=144 days), to measure loin and hind leg muscularity characteristics. These included *M. longissimus dorsi* (MLD) depth (D), width (W) and area (A) at the 5th lumbar vertebra (Jones *et al.*, 2002), width and depth of hind leg muscles (Jones *et al.*, 2002), and muscle volumes, bone lengths and muscularity indices in the loin (LRMV, lumbar spine length, LRMI) and hind leg (HLMV, femur length, HLMI) (Navajas *et al.*, 2007). After slaughter (average age=151 days), carcasses were subdivided into primal cuts (left and right legs, saddles and shoulders), which were then dissected to measure lean meat yield (LMY) and fat and bone weights in each cut. MLD was weighed separately. Linear model statistical analyses were performed (Genstat, 2006) to determine the effect of TM-QTL on each measured trait. TM-QTL carrier status, rearing rank (single or twin) and sex (female or castrate) were fitted as fixed effects, sire as a random effect, and age at scanning or slaughter as appropriate as a covariate.

Results Loin muscularity was significantly affected by TM-QTL in crossbred lambs (Table 1). UMD and CT-measured LRMV were significantly higher in TM-QTL carriers than non-carriers. Lumbar spine length and LRMI were not significantly affected by TM-QTL. Weight of MLD and LMY in the loin cut, and the percentage of the total carcass LMY that was contained in the loin region (%LMY in loin) were all significantly higher in TM-QTL carriers than non-carriers. There were few other significant effects of the TM-QTL either on ultrasound fat depth, weights of tissues in other carcass regions determined by dissection, or muscularity in the hind leg as assessed by CT scanning.

Table 1 Effect of TM-QTL carrier status on loin muscularity measured using ultrasound (US), CT and dissection

	US	CT scanning				Dissection			
	UMD (mm)	MLDW (mm)	MLDD (mm)	MLDA (cm ²)	LRMV (cm ³)	LRMI	MLD wt (g)	LMY in loin (g)	% LMY in loin
Non-Carrier	23.9	71.7	28.8	18.7	644	2.83	490	728	14.5
Carrier	24.8	73.0	30.6	19.5	670	2.88	522	780	15.4
sed	0.46	0.64	0.53	0.47	13.4	0.054	10.7	18.0	0.29
P-value	0.03	0.02	<0.001	0.09	0.04	0.23	<0.001	0.003	0.006
% difference	3.77	1.81	6.25	4.28	4.04	1.77	6.53	7.14	6.21

Conclusions TM-QTL has a significant effect on loin muscling characteristics of crossbred lambs carrying a single copy of the QTL compared to non-carrier lambs of the same cross. This confirms that the effect of TM-QTL on loin muscle depth in crossbred lambs is similar in magnitude to that originally identified in Texel sheep (Walling *et al.*, 2004). The smaller increase in MLD width compared to depth means that the increase in MLD area is slightly smaller than the increase in depth. There is no evidence from these data that TM-QTL affects other carcass traits. TM-QTL thus has potential to provide industry with a tool to increase carcass value through increased weight in the high value loin area. This project has also examined effects of TM-QTL on meat quality and animal health and welfare traits. The full results of ongoing analyses will enable recommendations to be made to the UK sheep industry on use of TM-QTL.

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Extending the Neutral Indel Model methodology to increase the proportion of exonic DNA found in the bovine genome

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Introduction The third build of the bovine genome comprises about 3 billion base pairs (bp; Ensembl, 2007). Locating functional DNA (fDNA) within this vast array of sequence is therefore a massive job. A method which can reduce the search space for this task may well have a wide application. It has been shown previously that the Neutral Indel Model (NIM; Lunter *et al.*, 2006) can be used to locate a high proportion of human (Lunter *et al.*, 2006) and bovine (Pollott, 2007) exonic DNA within a known 4% of the respective genomes. The NIM uses a mixture of methods from molecular evolution and comparative genomics to identify indel-purified segments (IPS) which should contain conserved fDNA. An IPS is the sequence located between two successive indels (insertions/deletions) and greater in length than a given threshold, as determined by input to the NIM method. Pollott (2007) found 64% of exonic DNA located in 233,715 IPS generated at a 0.1 false discovery rate (FDR) and these IPS overlapped with 87% of bovine genes. This increased to 81%, and 95%, respectively at a 0.5 FDR but the proportion of the genome searched increased from 3.3 to 10.7%. The objective of this work was to test some methods which may improve on the proportion of exonic DNA 'found' by the NIM without increasing the proportion of the genome searched by too much.

Materials and methods Build 2 of the bovine genome, Build 18 of the human genome, the bovine Ensgene file and the relevant Repeatmasker files were downloaded from the University of California website for this work (<http://hgdownload.cse.ucsc.edu/downloads.html>). The NIM software was supplied by its originator at <http://www.fgu.anat.ox.ac.uk/~gerton/software/Gapscan/> and modified to accommodate three extensions to the method. These were 1) include all inter-gap segments (IGS) found in given guanine and cytosine (G+C) windows (Lunter *et al.*, 2006); 2) allow codon gaps (gaps that were multiples of 3bp long, up to 39bp) and 3) extend IPS shoulders by 100 to 400bp either side. A range of parameters for each method was tried and their effectiveness measured by the proportion of exonic DNA located in the identified sequence, the proportion of IGS containing exonic DNA (IGSex) located and the proportion of the genome used.

Results Including all IGS located in the 250bp windows with the highest G+C content (G+C₂₀) increased the proportion of exonic DNA found by about 7.5% and the proportion of IGSex found by about 11%, at both 0.1 and 0.5 FDR levels. However, this resulted in having to search 10.7% and 13.3% of the genome respectively. Ignoring codon gaps resulted in a reduction in the amount of exonic DNA located due to a shift in the NIM regression line (not shown). Adding in the G+C₂₀ windows to the codon gap method resulted in a large increase in both the exonic DNA and IGSex found but, at the 0.5 FDR, required a search of nearly 20% of the genome to find them. Including successively lengthening shoulders around the IPS increased the amount of exonic DNA found but generally required a larger amount of the genome to be searched than the equivalent basic method results. The 'shoulders' method was tried at a 0.5 FDR but rapidly increased the amount of the genome scanned and so was not very effective.

Table 1 A summary of the hit rate of the various methods used to locate fDNA in the bovine genome using exonic DNA as the main indicator of fDNA (% of category).

Method	Exonic bp	IGSex	Genome used
NIM 0.1 FDR	63.9	34.7	3.3
NIM 0.5 FDR	80.6	48.7	10.7
NIM 0.1 FDR + G+C ₂₀	71.6	45.3	6.4
NIM 0.5 FDR + G+C ₂₀	88.1	60.7	13.3
Codon gaps 0.1 FDR	61.1	42.3	4.5
Codon gaps 0.1 FDR + G+C ₂₀	76.1	50.2	9.3
Codon gaps 0.5 FDR + G+C ₂₀	91.2	66.3	19.2
NIM 0.1 FDR + 100bp shoulders	66.1	42.7	5.7
NIM 0.1 FDR + 200bp shoulders	69.0	48.6	7.8
NIM 0.1 FDR + 300bp shoulders	70.8	51.5	9.8
NIM 0.1 FDR + 400bp shoulders	72.2	54.6	11.6

Conclusions Although the original NIM used at the 0.1 FDR looked promising as a way to find functional DNA in a small proportion of the bovine genome the extensions to the method failed to improve on the results. Most extensions increased the amount of exonic DNA located but the more successful methods also required a substantial increase in the proportion of the genome searched. The major drawback of the method appears to be the large number of small IGSex not 'found' by the NIM and further work is needed to improve its efficacy.

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Evaluation of the use of the Human Affymetrix GeneChip microarray for livestock species

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Introduction A microarray is an orderly arrangement of spots printed on an impermeable solid support, usually glass, silicon chips or nylon membrane. Each gene is represented by multiple spots and a single microarray chip can detect thousands of expressed genes simultaneously and in some cases the entire genome of an organism. Microarrays therefore allow high-throughput determination of changes in expression of thousands of genes of both known and unknown function in response to experimental treatment and so aids in the identification of novel genes associated with physiological pathways. For most mammalian species there is currently either a lack of a specific microarray or a limited coverage of the genome, both of which limit the use of microarrays in those species. For humans and rodents, however, there is complete coverage of the expressed genome. The aim of this study was therefore to determine whether the human Affymetrix GeneChip could be used to measure gene expression in a ruminant species and whether this is a viable alternative to species specific microarrays.

Materials and methods Initially, sheep genomic DNA was hybridised to the Human GeneChip array (Affymetrix - GeneChip® Human Genome U133 Plus 2.0) in order to identify cross-hybridising probes that can be used to analyse sheep RNA samples. To demonstrate differential gene expression between defined tissue types, RNA samples from muscle and liver from three sheep were analysed using the NASC Xspecies technique (Hammond *et al.* 2005). Data were analysed using GeneChip operating system (GCOS; Affymetrix), NASC Xspecies filter (<http://affymetrix.arabidopsis.info/xspecies/>) and Genespring GX (Agilent).

Table 1 Relative expression of selected genes in sheep muscle and liver

Gene	Expression Muscle	Expression Liver	Fold change (muscle/liver)
Ryanodine receptor 1 (skeletal muscle)	2.4251	0.0143	169.3
Myosin, light	2.2790	0.0169	134.5
Myosin, heavy	2.0667	0.0208	99.31
Troponin C2, fast	2.1411	0.0420	51.00
Calpain 3, p94	2.1375	0.0466	45.87
Muscle type creatine kinase	2.0222	0.0635	31.85
Cytochrome P450	0.6535	1.3090	0.499
Stearoyl-CoA desaturase	0.7146	1.4994	0.477
Hepatocyte nuclear factor 4 alpha	0.6730	1.4226	0.473
Hepatocyte growth factor	0.5799	1.3582	0.427
Liver type pyruvate kinase	0.0949	1.9357	0.049
Pantothenate kinase 1	0.0488	2.3185	0.021

Results and Discussion There was a high level of retained probes between the Human and Sheep genomes. At least 30,000 genes could be measured in sheep muscle and/or liver using the human specific microarray. Table 1 shows relative expression of muscle and liver specific genes within the sheep tissues. A fold change greater than 1 indicates a gene was more highly expressed in muscle, and a fold change less than 1 indicates more highly expressed in liver. As expected muscle specific genes such as myosin were more highly expressed in muscle whereas liver type pyruvate kinase, for example, was more highly expressed in liver. As a consequence of using well-annotated Human arrays it will also be possible to look at expression of genes involved in specific pathways in sheep (e.g. muscle fibre type proteins or signalling cascades). Hence it will not be necessary to develop similar technologies specifically for sheep studies. The ability to use cross-species hybridisations for mammals is in agreement with previously published studies using this same technique for different species of plant (Hammond *et al.* 2005). Although for any particular gene not all the probes hybridised, there were sufficient to make the use of this technology applicable and viable for sheep studies and it is likely that the same will be true for other livestock species such as pigs and cattle.

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Leptin, growth hormone receptor and DGAT1 gene polymorphisms in Holstein cattle

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Introduction DNA polymorphisms have been well established for the diacylglycerol acyltransferase 1 (DGAT1), leptin and growth hormone receptor (GHR) genes, and they seem to correlate with several economically important traits in dairy cattle. In particular, a lysine-to-alanine substitution (K232A) and a phenylalanine-to-tyrosine substitution have been described for the DGAT1 (Kaupe *et al.*, 2004) and GHR genes (Aggrey *et al.*, 1999, Blott *et al.*, 2003), respectively. Polymorphisms have also been identified within the leptin gene; for example, one has been found in an intronic sequence (RFLP1) and another inside exon 3 (Liefers *et al.*, 2002). The objective of the present study was to determine the polymorphism status of these three gene loci in Holstein cows raised in Greece.

Materials and methods DNA was extracted from blood samples collected from 319 Holstein cows which were raised in a large commercial dairy farm located in Northern Greece. DGAT1 and leptin (RFLP1) genotyping was performed using a PCR-RFLP method according to previous studies (Kaupe *et al.*, 2004, Liefers *et al.*, 2002, Pomp *et al.*, 1997). The GHR gene polymorphism was investigated with an ARMS-PCR method developed in our laboratory. The following primers were used: 1) for DGAT: DGAT-F 5'-GCACCATCCTCTTCCTCAAG-3', DGAT-R 5'-GGAAGCGCTTTCGGATG-3', 2) for Leptin: LEPT-F 5'-TGGAGTGGCTTGTTATTTTCTTCT-3', LEPT-R 5'-GTCCCCGCTTCTGGCTACCTAACT-3' and 3) for GHR: AS1 5'-GGGCTAGCAGTGACATTATA-3', AS2 5'-GGGCTAGCAGTGACATTATT-3', COM 5'-ACCTCTGGTCTGGAATAAA-3'. PCR and RFLP products were electrophoresed on agarose gels and visualized under UV light. Allelic (polymorphism) frequencies were estimated with a count of actual genotypes. A chi-square test comparing observed and expected genotypic frequencies was performed to assess the Hardy-Weinberg equilibrium status of the population sample.

Results The sizes of the PCR and RFLP products are shown in Table 1. Percentages of homozygous normal (N/N), heterozygous (M/N) and homozygous mutant (M/M) genotypes as well as allelic frequencies in each locus are presented in Table 2. Leptin and GHR loci were found to be in Hardy-Weinberg equilibrium ($P > 0.05$) whereas DGAT1 was not ($P < 0.05$).

Table 1 PCR and RFLP product sizes

	PCR product (bp)	RFLP products (bp)		
		Homozygous normal	Heterozygous	Homozygous mutant
DGAT1	411	411	411, 205, 206	205, 206
Leptin	400	400	400, 300, 100	300, 100
GHR	341	-	-	-

Table 2 Number of genotypes and genotypic (N/N, M/N, M/M) and allelic (N, M) frequencies (%) in the three loci

	No. Genotypes	N/N	M/N	M/M	N	M
DGAT1	309	23.6 (n=73)	76.4 (n=236)	0 (n=0)	61.8	38.2
leptin	314	78.3 (n=246)	21.7 (n=68)	0 (n=0)	89.2	10.8
GHR	318	75.2 (n=239)	23.9 (n=76)	0.9 (n=3)	87.1	12.9

Conclusion Results showed that all three loci were polymorphic in the studied population. However animals homozygous for the mutant allele (M/M) were not identified for either DGAT1 or leptin genes. In contrast, homozygous mutants were found for the GHR gene. Lack of Hardy-Weinberg equilibrium in the DGAT1 locus suggests possible effects of intense indirect selection. These data are currently being investigated for possible associations with clinical, biochemical and reproductive parameters.

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Linkage disequilibrium between single-nucleotide polymorphism in 5'-flanking region and the coding part of bovine beta-Lactoglobulin gene in three native cattle and Holstein breeds of Iran

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Introduction Beta-lactoglobulin (LGB) is the best studied protein and found in whey protein milk of ruminants. It has been observed that the heterozygous cows produce LGB-A much more than LGB-B (Wagnet *et al.* 1994, Gustafson *et al.* 2003). The differential expression of LGB-A and LGB-B has been suggested to be because of polymorphisms in the regulatory region of LGB, and where these promoter alleles occur in linkage distinct with the alleles in the coding part of the gene (Gustafson *et al.* 2003). In this study pyrosequencing and PCR-RFLP were utilized to estimate allele frequencies at positions -435(R10), -424(R9), -422(R8) and coding region at codon 64, and also linkage disequilibrium in three native breeds (Mazandarani, Sarabi and Golpaygani) and Holstein cattle of Iran.

Materials and methods Blood samples were collected from 100 Mazandarani (M), 90 Sarabi (S) and 112 Golpaygani (G) native cattle breeds and 110 Holstein randomly (H). M and G breeds are considered as *Bos indicus* and S breed as *Bos taurus*. DNA extraction and amplification of coding part of LGB gene (exon IV) was conducted as described by Zakizadeh *et al.* (2007). Pyrosequencing method was used to sequence R8 and R9 variants of mammary cell activating factor (MAF) and also R10 variant located in AP-2 binding site of 5'-region and partial of exon I (Wagner *et al.*, 1994). A specific primer pair was designed to amplify a 186 bp fragment of promoter region from nucleotide 285 to 470 (GenBank, X63139) by pyrosequencing Software. DNA was amplified in a total volume of 50 µl containing 100 ng genomic DNA, 0.2 µM of each primer, 0.2 mM dNTP, 3.5 mM MgCl₂, 1X PCR buffer and 1 U Taq DNA polymerase (Genaxxon, Germany). PCR conditions were 94 Celsius degree for 1 minute, 62 Celsius degree for 30 seconds and 72 Celsius degree for 40 seconds with 35 cycles and finally 72 Celsius degree for 5 minutes. The pyrosequencing was prepared after standard protocol with Pyro Gold Reagents using the PSQ™96MA System (Biotage, Uppsala, Sweden). Haploview software V.3.32 was used to estimate recombination rates and haplotypic frequencies in each breed separately

Results Allele-A frequency of promoters and coding region are given in Table 1. There was different patterns of genotype combination between 5'-flanking and coding regions in each breed (Table 2). We found also high linkage disequilibrium between polymorphism at position -435 and codon region in Holstein (LOD=4.88).

Conclusion Wagner *et al.* (1994) reported that the 'A' allele of coding region of LGB was mostly associated with the B alleles within the promoter sequence. In our study, approximately 57 percent of all breeds which had the LGB-A variant were combined with B allele at two positions R10 or R8. The exception was at position R9, which based on Wagner *et al.* (1994) T mutation had been named as allele A, although it had lower frequency than their assumption (they had supposed the most frequent allele as allele A). Therefore LGB-B variant was combined approximately in 70 percent of samples with allele R9-B and not in combination with allele A of R9 mutation at position -422.

Differences between allele frequencies and haplotype patterns of our studied breeds could be due to different origin, number of samples, and/or the effect of natural selection in native breeds, or the name of alleles at position R9, which Wagner *et al.* (1994) had considered the most frequent variant as A allele. Additional haplotypes could be expected exist in this study which result in more genotype combination that much more samples would be necessary.

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Table 1 Allele frequency of 'A' variant in three mutations sites of 5' and coding regions of LGB promoter *

Name	Position (Nt.)	Frequency of allele A in breeds **			
		M	S	G	H
R8	-422(G)	0.83(81)	0.59 (74)	0.9 (82)	0.76 (96)
R9	-424(T)	0.07(68)	0.05 (62)	0.04(70)	0.01 (100)

Table 2 Genotype combination percent of LGB-B variant with allele A of promoter regions at R10, R9, and R8 in studied breeds

Breed	Polymorphic sites of promoter		
	R10	R9	R8
M	57	70	62
S	56	62	38
G	62	74	67
H	62	73	48

Selection, characterisation and screening of lactic acid bacteria of chicken origin that have probiotic properties

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Introduction Increasing scientific awareness of health and production promoting intestinal bacteria has enhanced the use of probiotic bacteria as active functional ingredients in animal and human nutrition. Strict selection criteria, suggested by Edens (2003), for potential probiotics in order to achieve well established and positive probiotic effects have been engaged, including the safety for the host and the capacity of the strains to be viable as well as metabolically active within the gastrointestinal tract (GI). *In vitro* methods have been used for screening potential probiotic strains using a dynamic model that mimics *in vivo* GI conditions as closely as possible. The objective of this study was to isolate, characterise and further select beneficial lactobacillus strains in an attempt to predict candidates that could be used *in vivo* as chicken probiotic adjuncts.

Materials and methods A total of 53 lactic acid bacteria (LAB) isolated from the contents of the crop, caecum and small intestine and from the mucosa of the crop, jejunum and ileum of three organically farmed chickens were studied for their potential use as probiotic feed supplements. After the isolation of the LAB *in vitro* studies have been conducted including homo/heterofermentation test (Müller 1990), autoaggregation and coaggregation studies with enteropathogenic bacteria (Drago *et al.* 1997; Kmet and Lucchini 1999), adhesion activity namely; mucus binding assay and adhesion to chicken intestinal epithelial cells (Garriga *et al.* 1998). Additionally, the antagonistic activity against several enteropathogens (Olnood *et al.* 2007) as well as the tolerance to the acidic pH and bile salts of the GI tract, were tested, using a simulation of the digestive process. The data was subjected to ANOVA using the General Linear Model MINITAB v.14.0. Aggregation and adhesion results were confirmed by Light and Scanning Electron Microscopy (EM). The strains that showed a potential probiotic effect were identified using API CHL kit (BioMérieux, UK).

Results From 53 LAB that were tested for their capacity to aggregate, 20 were non-aggregative. Eleven bacteria showed a rapid autoaggregation, 12 LAB had a normal reaction and the rest of the strains showed weak autoaggregation activity. The 23 LAB that showed normal and rapid aggregative activity were further tested for their ability to coaggregate with *S. enteritidis*, *Clostridium perfringens*, *Escherichia coli* and *Salmonella typhimurium*. One LAB strain identified as *Lactobacillus plantarum* showed maximum aggregation (Figure 1), two that have been identified as *Lactobacillus salivarius* showed marked aggregation, six showed good aggregation, nine partial aggregation and three showed no, or almost no aggregation. Of the LAB tested, *L. plantarum* and *L. salivarius* had the best inhibitory activity against enteropathogens. Also, both of the strains proved to have enhanced adherent capacity to epithelial cells, with the *L. salivarius* being better than the *L. plantarum* (Figure 2, 3). All of the LAB tested survived transit through the stimulated GI tract and were found to be equally resistant to the acidic environment ($P > 0.05$).

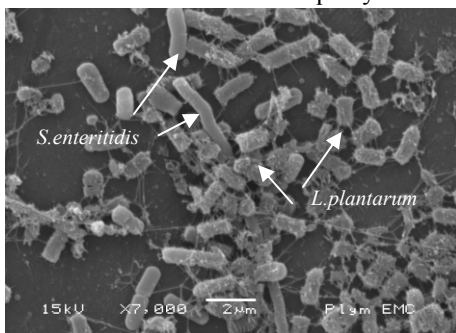


Figure 1 Aggregation of *L. plantarum* with *S. enteritidis* (Scanning EM)

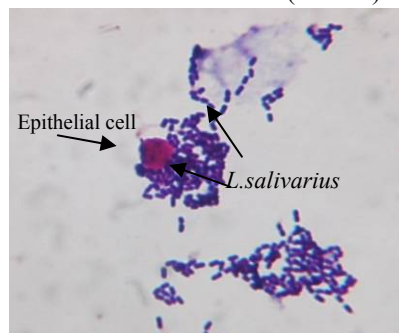


Figure 2 Adherence of *L. salivarius* to chicken epithelial cell (Light EM)

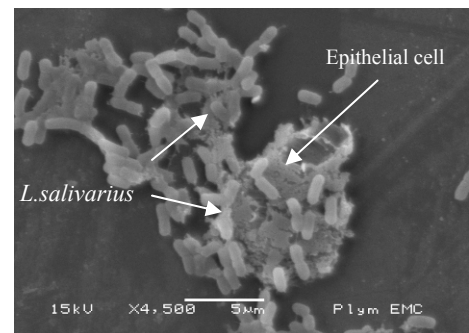


Figure 3 Adherence of *L. salivarius* to chicken epithelial cell (Scanning EM)

Conclusion The results indicate that two strains of lactobacilli *L. plantarum* and *L. salivarius* isolated from the natural gut microflora of poultry exhibited strong potential as probiotic adjuncts and could perform effectively within the GI tract. The use of isolated and purified LAB from the GI tract of chickens provides an alternative to the use of ileal extracts as a means of introducing beneficial bacteria to the digestive tract of chicks as it circumvents the problems of potential introduction of pathogenic organisms associated with the use of ileal extracts. However, given the complexity of the chicken GI tract, the proof of efficacy of the two probiotic bacteria in broilers will require *in vivo* studies.

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Inbreeding effects on economic traits of native chickens of Fars Province

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Introduction Native chickens are important in some rural areas. They usually produce meat and eggs without extra feed using only picked food. Improving their economical traits, such production efficiency would save these genetic resources. Kianimanesh (2002) showed that age at sexual maturity, number of eggs, egg weight and body weight at 8th week of age are among most important traits for improving economical efficiency of Iranian native fowl. Although, natural selection is the great evolutionary force that fuels genetic change in all living things, selection at the long term has important responses such as increase of inbreeding and decrease genetic variation. However, changes in average of production traits and population response in breeding programs after a few generations of selections depended on accuracy and intensity of selection, effective size of population and the rate of inbreeding. The aim of this research was to study the rate of additive inbreeding on some of the economical traits in Fars native chickens in Iran.

Materials and methods Five generations of data from a poultry breeding program were provided by the Center of investigation on native poultry Jihad Keshavarzi Organization of Fars Province. The birds originally were collected from different area of the Fars province. Selection procedures for these birds were on the basis of estimated breeding value and calculated genetic and environmental parameters using BLUP in an animal model. A pedigree file of 14,520 birds were used to calculate the rate of inbreeding coefficient and the influence of the rate on body weight at day old (BW1), body weight at 8th weeks of age (BW8), body weight at 12 weeks of age (BW 12) and the weight of first egg produced (EGGW1), egg weight at 30 weeks of age (EGGW30) and the average number of stock eggs per day (EGG/DAY). Mean of inbreeding coefficient for each generation was calculated. A multiple regression procedures were employed to calculate the effects of inbreeding on different recorded production traits using SAS software.

Results Overall, 34.5 %of birds in the population were inbred with the average inbreeding coefficient of 0.028. The range of inbreeding coefficients for inbred birds were from 0.0019 to 0.15. Figure 1 showed the cumulative rate of inbreeding with body weight at day old (BW1) over five generations of proceed breeding plan. The average calculated inbreeding effect on BW1, BW8 and BW12 were -0.09, 0.06, and 0.018 respectively. While the average calculated inbreeding effect on EGGW1, EGGW30 and EGG/DAY were -0.03, -0.12, and 0.00016 respectively. These results showed that per percent of increase in the rate of inbreeding coefficient there would be a decrease of 0.9 gr in BW1, 0.03 gr in EGGW1 and 0.12 gr in EGGW30 ($p < 0.001$). Inbreeding regression equation on generation in different traits also used to calculate relationship between inbreeding and these two group of traits:

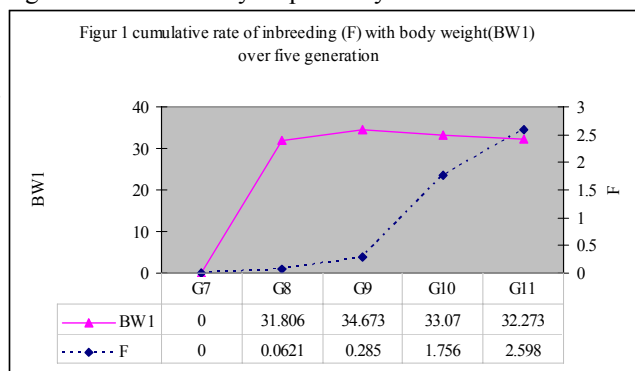
$$Y_1 = A + b_1(F) + b_2(In)$$

$$Y_2 = A + b_1(F) + b_2(In) + b_3(Age)$$

Where Y_1 is the vector of observed values of the weight traits and Y_2 is the vector of observed values of the egg traits b_1, b_2 and b_3 regression coefficient of generation, inbreeding and age of sexual maturity respectively.

Table 1 Distribution of inbreeding coefficients across generations

Generation	No.of records	Ave	Min	Max	Standard deviation (Sd)
G7	2731	0	0	0	0
G8	2817	0.062	0	12.5	0.88
G9	2997	0.285	0	12.5	1.408
G10	2985	1.76	0	7.03	1.55
G11	2981	2.6	0	15.04	1.58



Conclusion In designing a breeding programme, the number of individuals producing the next generation could effect stock performance. Also, in evaluating of breeding programme someone should calculate the effect of inbreeding rate on performance traits in order to avoid misjudgements on the amount of progress in economical traits.

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Study of genetic parameters for economic traits in Iranian native fowl

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Introduction Iranian native fowls are raised for both meat and egg yield. Growth rate and egg production under conventional rearing systems in the villages are very low, Also, in past several decades imports of exotic breeds have increased the risk of extinction native fowl species. Evidence shows that there is good and useful variation in native fowl species for different traits, especially for those of economic importance. The objective of the current study is to estimate genetic parameters for economic traits of Iranian native fowl, so that efficient multi-trait selection programmes can be developed which will maximise economic return and secure genetic diversity.

Materials and methods Data were collected between 1988 to 2006 and consisted of body weight (BW) at 8 weeks for Mazandaran station or 12 weeks for other stations, age at first egg (AFE), egg number laid at 12 first weeks of production (EN₁₂) and mean egg weight between 28 to 32 weeks (MEW) related to four stations (Mazandaran, Fars, West Azarbaijan and Esfahan). These data were used for estimation of co(variance) components by using derivative free restricted maximum likelihood algorithm (Meyer, 1991) and following multiple traits animal model: $y_i = X_i b_i + Z_i u_i + e_i$ Where y was the vector of observations in i^{th} trait, b_i was the vector of fixed effects (b_1 for BW included generation, hatch and sex, b_2 for EN₁₂ included generation, hatch and days in production (DP) as covariate and b_3 and b_4 for AFE and MEW included generation and hatch), u_i was the vector of random additive genetic effects of animals, e_i was the vector of random residual effects and X and Z were incidence matrices relating fixed and random effects to the records, respectively.

Results Results showed that most heritabilities that were estimated, except EN₁₂ (0.099 - 0.185), were greater than 0.20 (Table 1). The highest estimated heritabilities for all traits were related to Fars station, whereas most heritabilities in West Azarbaijan was less than other stations, agreeing with Nejati *et al* (2002). The estimated heritabilities of BW were medium to high and varied from 0.228 for Esfahan to 0.548 for Fars, whereas, its values for MEW were higher and ranged from 0.223 to 0.638 for West Azarbaijan and Fars, respectively. The heritability for AFE was estimated 0.270 for Esfahan to 0.520 for Fars. The heritability for EN₁₂ was estimated at 0.158, 0.322, 0.099 and 0.185 for Mazandaran, Fars, West Azarbaijan and Esfahan, respectively. Also the estimated heritabilities reported by Francesch *et al* (1997) for egg number and egg weight were 0.20 and 0.59, respectively. Most of the estimated genetic correlations, except between BW and EW and between AFE and EW, were negative and agreed in sign with those reported by several (e.g., Zieba *et al.*, 2003). The genetic correlation between BW and EN₁₂, except Fars (0.172), was negative (-0.111 to -0.225). AFE had a medium to high negative correlation with EN₁₂ ranging from -0.384 to -0.987 for Mazandaran and Fars, respectively.

Table 1 The estimated heritabilities \pm standard errors of traits for stations

Trait	Mazandaran	Fars	Azarbaijan	Esfahan
BW	0.279 \pm 0.009	0.548 \pm 0.014	0.254 \pm 0.014	0.228 \pm 0.014
AFE	0.346 \pm 0.012	0.520 \pm 0.014	0.276 \pm 0.027	0.270 \pm 0.021
EN ₁₂	0.158 \pm 0.009	0.322 \pm 0.012	0.099 \pm 0.018	0.185 \pm 0.019
MEW	0.458 \pm 0.012	0.638 \pm 0.014	0.223 \pm 0.021	0.246 \pm 0.022

Table 2 The estimated genetic correlations \pm standard errors of traits for stations

Correlated traits		Mazandaran	Fars	Azarbaijan	Esfahan
BW	AFE	-0.104 \pm 0.029	-0.116 \pm 0.026	-0.047 \pm 0.012	-0.039 \pm 0.014
	EN ₁₂	-0.143 \pm 0.037	0.172 \pm 0.027	-0.111 \pm 0.087	-0.225 \pm 0.061
	MEW	0.417 \pm 0.024	0.518 \pm 0.018	0.184 \pm 0.058	0.294 \pm 0.054
AFE	EN ₁₂	-0.384 \pm 0.033	-0.987 \pm 0.003	-0.748 \pm 0.069	-0.675 \pm 0.037
	MEW	0.210 \pm 0.026	0.085 \pm 0.023	0.137 \pm 0.075	0.251 \pm 0.061
EN ₁₂	MEW	-0.264 \pm 0.033	-0.186 \pm 0.025	-0.199 \pm 0.101	-0.470 \pm 0.066

Conclusions Results show that the different estimated parameters is caused by different genetic and population structure of birds in each station such as number of birds, number of generations and initial potential of each breed at the beginning of project. Between traits the highest ratio of genetic variation (heritability) is related to MEW and between stations Fars has more value in comparison with other stations.

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Effects of vitamin E and selenium dietary supplements on performance, egg quality and immune response of laying hen exposed to high environmental temperature

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Introduction High environmental temperature is one of the important stressors faced by laying hens usually in summer months. In a hot environment, hens exert extra effort to maintain their body temperature within a normal range. This challenge is associated with behavioural, physiological, hormonal and molecular responses. Hens in stressful environments decrease their rate of ovulation and also produce lower antibody response to a variety of antigens. Vitamin E and selenium have important roles in the antioxidant system and they have an influence on the immune system. Dietary supplementation with Vitamin E facilitates release of vitellogenine necessary for yolk formation which reduces its release in heat stress (Bollengier-Lee *et al.*, 1998). Also, Chickens provided with organic selenium are more resistant to thermal stress than chickens fed inorganic sodium selenite (Mahmoud and Edens, 2003).

This study was conducted to determine the effect of dietary vitamin E and selenium, as well as comparison of organic and inorganic selenium, on performance, egg quality and antibody response to sheep red blood cells (SRBC) in laying hens exposed to high environmental temperature.

Materials and methods One hundred and forty four single comb white leghorn (Hy-line W 36 strain) chicks, were randomly divided into two houses with different environmental temperatures and six dietary treatments (control, 0.4 ppm sodium selenite (SS), 0.4 ppm selenium yeast (SY), 200 ppm dl-alpha-tocopheryl acetate (α TA), 0.4 ppm SS + 200 ppm α TA and 0.4 ppm SY + 200 ppm α TA). During the experiment, hens were exposed to cycling heat stressing one of the houses. The temperature and relative humidity of the first house was 24 ± 2 °C and 50 ± 10 %, respectively. In the other house temperature and relative humidity were increased for 5 h/day to 33 ± 2 °C and 45 ± 11 %, respectively. Egg production, egg weight and feed consumption were recorded during the 7-week study. Egg quality characteristics including: shell thickness, shell resistance, shell weight, albumen quality, yolk weight, yolk and albumen pH were measured every two weeks. Antibody production against SRBC was used to assess the humoral immune response. Results were statistically analyzed by ANOVA with the general linear models procedure of SAS[®] software. Means were compared using Duncan's multiple-range test, and significance was determined at $P \leq 0.05$. The design was a $2 \times 3 \times 2$ factorial arrangement in which replicates were considered to be cages of hens (four hens/cage \times three replications) fed the same dietary regimen.

Results As can be seen from Table 1, most production parameters were affected by high environmental temperature. Egg weight, shell weight, shell thickness, and yolk weight were all significantly decreased when the hens were exposed to heat stress. Selenium and vitamin E did not show any significant effect on hen performance and eggs quality characteristics. The primary and secondary antibody titers to SRBC were significantly lower in the hens exposed to high environmental temperature. The secondary antibody production was significantly higher in hens fed the additional amount of vitamin E as compared to controls ($P < 0.05$). However, selenium supplementation also increased antibody titers to SRBC but these differences were not statistically significant.

Table 1 effect of treatments on the performance of hens and antibody production against SRBC

Treatments	Egg production (% hen day)	Egg weight (g)	Egg mass (g)	Feed consumption (g/hen/day)	FCR (g/g)	SRBC 1 st titer	SRBC 2 nd titer
Temperature							
Normal	72.87 ^a	61.5 ^a	44.1 ^a	109.6 ^a	2.52	6.6 ^a	9.2 ^a
Heat stress	68.37 ^b	59.7 ^b	41.2 ^b	105.9 ^b	2.62	5.1 ^b	7.8 ^b
Selenium							
0	71.00	60.6	42.8	107.2	2.54	5.2	8.1
0.4 SS	71.09	60.5	42.4	109.1	2.62	6.2	8.8
0.4 SY	69.76	60.4	42.6	107.0	2.56	6.1	8.7
Vitamin E							
0	70.94	60.6	42.5	107.7	2.58	5.6	8.0 ^b
200	70.31	60.6	42.8	107.8	2.56	6.1	9.1 ^a
SEM	1.015	0.28	0.61	0.70	0.038	0.27	0.29
Temperature	*	**	*	*	NS	**	*
Selenium	NS	NS	NS	NS	NS	NS	NS
Vitamin E	NS	NS	NS	NS	NS	NS	*
Interactions	NS	NS	NS	NS	NS	NS	NS

^{a,b} Means in the same column within a variable with different superscripts differ significantly * ($P < 0.05$), ** ($P < 0.01$), NS: not significant

Conclusions Results of this experiment confirm that high environmental temperature caused weak performance, egg quality and antibody response of hens. Diet supplementation with selenium did not affect any of this parameters but vitamin E addition to the diet, in the form of α -TA, significantly improved antibody production in laying hens.

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Efficacy of enzyme extracts from fungi and a commercial feed enzyme for improving the nutritional value of palm kernel cake for broiler feeding

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Introduction Broilers are unable to utilize palm kernel cake (PKC) efficiently because it contains high amounts of non starch polysaccharides (NSPs) and broilers do not have the full complement of the endogenous enzymes required to break down the NSPs to soluble carbohydrates. Microbial enzymes have been reported (Bachtar, 2005) to be able to act on both the endo and exo sites of NSPs, breaking both the β -1,4 and β -1,6 linkages with the production of soluble carbohydrates. Multi enzyme complexes from fungi have the potential to breakdown the NSPs in PKC thereby enhancing its nutritive value for broiler feeding. It was the aim of this study to apply enzyme extracts from *Aspergillus niger*, *Trichoderma viride*, *Rhizopus stolonifer* and *Mucor mucedo* on PKC and to investigate the effect of the biodegraded PKC on performance of broilers.

Materials and methods Purified enzyme extracts were obtained from *A. niger*, *T. viride*, *R. stolonifer* and *M. mucedo* and applied on autoclaved PKC at the rate of 250ml kg⁻¹. A commercial enzyme, Roxazyme G2G with the ability to degrade NSPs was also applied on autoclaved PKC at the manufacturer's recommended rate of 150g tonne⁻¹. The enzymic application was for 7 days after which the samples were analysed for proximate composition (AOAC, 1995), hemicellulose and cellulose (Van Soest, 1995) and soluble sugars (Somogyi, 1945). Six diets were formulated with Diet 1 containing 70 g kg⁻¹ of unbiodegraded PKC. Diets 2, 3, 4 and 5 consisted of 70g kg⁻¹ of the PKC biodegraded with *A. niger*, *T. viride*, *R. stolonifer* and *M. mucedo*, respectively and Diet 6 consisted of 70 g kg⁻¹ of unbiodegraded PKC supplemented with Roxazyme G2G a commercial feed enzyme produced from fungi and used by poultry farmers in Nigeria. All the diets were formulated with the addition of other ingredients such that they were isonitrogenous and isoenergetic to supply the required nutrients for broiler starters and finishers. Two hundred and forty 1-d-old broilers were weighed and 40 birds were randomly distributed to the diets with each diet having 4 replicates of 10 birds each. On d 28, the diets were switched to finisher diets with the amount of the undegraded PKC in diets 1 and 6 and degraded PKC in Diets 2, 3, 4 and 5 increased to 100 g kg⁻¹. They were offered the diets and water *ad libitum* for a further 28 days. Feed intake and weight gain were recorded weekly and data analysed using ANOVA procedures in SPSS 11.0 for Windows (SPSS Inc., Chicago, IL, USA). Differences between means were separated using the Duncan's Multiple Range test (Steele and Torrie, 1980).

Results Highest crude protein, phosphorus and ME values were 21.2g kg⁻¹ with *T. viride*, 0.95g kg⁻¹ with *R. stolonifer* and 10.4MJ kg⁻¹ ME with *M. mucedo*, respectively (Table 1). Soluble sugars in the biodegraded PKC, increased significantly ($p < 0.05$). Birds on the Diets with biodegraded PKC had better ($p < 0.05$) performance than those on the other diets (Table 2).

Table 1 Chemical composition and energy content of unbiodegraded and enzyme treated palm kernel cake

Parameters	Undegraded PKC	PKC +An	PKC +Tv	PKC +Rs	PKC +Mm	PKC +RG2G	SEM	P value
Crude protein	12.008 ^c	20.7 ^a	21.2 ^a	19.6 ^{ab}	18.6 ^b	17.8 ^b	1.85	0.001
Phosphorus	0.50 ^c	0.92 ^a	0.85 ^{ab}	0.96 ^a	0.77 ^b	0.71 ^b	0.04	0.0004
Hemicellulose	24.0 ^a	22.2 ^b	18.2 ^c	14.3 ^d	19.0 ^c	23.1 ^a	1.33	0.004
Cellulose	16.6 ^a	8.16 ^c	11.6 ^c	14.5 ^b	13.7 ^d	14.8 ^b	1.02	0.0016
Glucose	197.54 ^c	957.76 ^a	352.41 ^c	334.21 ^c	654.59 ^b	245.36 ^d	0.07	0.001
Fructose	120.03 ^d	256.50 ^a	108.04 ^b	122.40 ^b	134.80 ^b	860.11 ^c	0.04	0.001
Galactose	100.34 ^c	872.71 ^a	210.50 ^c	420.30 ^b	760.80 ^a	140.02 ^d	0.01	0.0242
Sucrose	34.60 ^c	224.26 ^a	104.67 ^c	106.88 ^c	176.89 ^b	54.75 ^d	0.04	0.0001
ME (MJ kg ⁻¹)	8.94 ^d	9.59 ^b	9.55 ^b	9.60 ^b	10.4 ^a	9.37 ^c	1.18	0.0067

An = *A. niger*, Tv = *T. viride*, Rs = *R. stolonifer* and Mm = *M. mucedo*

Table 2 Performance of broiler finishers fed diets with undegraded and degraded palm kernel cake-based diets

Parameters	Undegraded PKC	PKC +An	PKC +Tv	PKC +Rs	PKC +Mm	PKC +RG2G	SEM	P Value
Daily feed intake (g)	110.82 ^d	136.48 ^a	130.65 ^b	128.58 ^b	120.41 ^c	115.65 ^{cd}	2.67	0.0022
Weight gain (g)	44.08 ^d	52.83 ^a	50.58 ^b	49.57 ^b	48.00 ^c	44.18 ^d	2.85	0.023
FCR	2.51	2.58	2.58	2.59	2.51	2.62	0.51	0.310

An = *A. niger*, Tv = *T. viride*, Rs = *R. stolonifer* and Mm = *M. mucedo*

Conclusion Results suggest that a broad spectrum enzyme complex capable of enhancing the nutritive value of PKC for broiler production can be obtained from the four fungi investigated

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Effects of yeast cell wall and sodium bentonite on antibody titres of broiler chickens fed ration containing aflatoxin

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Introduction Aflatoxins (AFs), potent mycotoxins produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus*, are a major concern in poultry production. AF contamination causes reduced animal efficiency, increased susceptibility to environmental and microbial stresses and reduction of immune function (Miazzo *et al.*, 2000). Different approaches such as the use of mold inhibitors, fermentation, physical separation, irradiation, ammoniation and ozone degradation have been used to prevent food contamination by AFs. Unfortunately, most of these procedures are expensive, time-consuming and only partially effective. Currently, the most promising and practical approach has been the addition of adsorbents (Mycozorb and aluminosilicates) to contaminated feeds to selectively bind the aflatoxin molecule. So far, the effects of aflatoxin adsorbents have not been evaluated on antibody titres of chickens. The purpose of this study was to evaluate the effects of sodium bentonite and a commercial yeast cell wall product (MycosorbTM) on antibody titres against Newcastle and Infectious bursal diseases of chickens fed rations containing AF.

Materials and methods High levels of aflatoxins were produced on rice as a natural substrate by toxigenic *A. parasiticus* according to the method of Shotwell *et al.* (1966). Four hundred and eighty 1-day-old male broilers (Ross 308), obtained from a commercial hatchery, were used in this study. Individually weighed chicks were divided at random into 6 groups. There were 4 replications of 20 broiler chicks for each dietary treatment. Basal diet was prepared and formulated to contain National Research Council (1994) requirements of all nutrients. The experimental design consisted of 6 dietary treatments. (1) negative control: basal diet; (2) positive control (AF diet): basal diet plus 1 mg AF per kg diet; (3) AF diet containing 0.5% sodium bentonite; (4) AF diet containing 1.0% sodium bentonite; (5) AF diet containing 0.05% yeast cell wall; (6) AF diet containing 0.1% yeast cell wall. Yeast cell wall (MycosorbTM) was provided from Alltech, K.Y., USA. Birds were vaccinated against Newcastle and infectious bursal on 3rd and 21st day of experiment. Blood samples were collected from the wing vein of three birds per replicates at the age of 21, 28, 35 and 42 day, sera were separated and used for measurement of antibody contents. Data were analyzed as CRD design by the GLM procedure of SAS (1996). All statements of significance are based on the probability level of 0.05.

Results The geometric means of antibody titre against Newcastle and infectious bursal diseases at 21st, 28th, 35th and 42nd day of different groups are in Table 1. The highest titre ($P < 0.05$) was observed in negative control group and the lowest titre ($P < 0.05$) was observed in positive control group, for both antibodies. The aflatoxin causes the regression of the Bursa of Fabricius, so the low titre against both diseases may be attributed to the regression of Bursa of Fabricius.

Table 1 Geometric mean titre of Newcastle and infectious bursal diseases

Sodium bentonite and yeast cell wall improved ($P < 0.05$) antibody titres. In some instances, yeast cell wall further improved the titres compared with sodium bentonite. The results of this study are in line with Ibrahim <i>et al.</i> (2000) who reported that addition of sodium bentonite was significantly effective in ameliorating the negative effect of AF on the percentage and mean of phagocytosis.	Treatments			
	Absorbent	21st day	28th day	35th day
<i>Newcastle</i>				
Negative control	6.7 ^a	7.6 ^a	8.8 ^a	9.2 ^a
Positive control	2.9 ^c	4.2 ^d	4.7 ^d	4.8 ^d
Sodium bentonite 0.5%	4.2 ^b	6.7 ^b	6.8 ^b	8.0 ^b
Sodium bentonite 1.0%	4.1 ^b	6.7 ^b	5.8 ^c	6.8 ^c
Yeast cell wall 0.05%	4.3 ^b	6.2 ^c	6.1 ^c	6.6 ^c
Yeast cell wall 0.1%	3.9 ^b	7.3 ^a	6.6 ^b	6.3 ^c
SEM	0.28	0.19	0.22	0.36
<i>Infectious bursal diseases</i>				
Negative control	369 ^a	1035 ^a	1795 ^a	2396 ^a
Positive control	307 ^c	569 ^c	1566 ^c	2193 ^c
Sodium bentonite 0.5%	362 ^a	1005 ^a	1605 ^{bc}	2262 ^{bc}
Sodium bentonite 1.0%	326 ^b	680 ^b	1583 ^{bc}	2208 ^c
Yeast cell wall 0.05%	356 ^a	1010 ^a	1618 ^{bc}	2312 ^{ab}
Yeast cell wall 0.1%	360 ^a	1023 ^a	1644 ^b	2383 ^a
SEM	12.2	27.7	41.5	63.1

Means within a column with different superscripts are significantly different ($P < 0.05$)

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Effect of concentration of phenolic compounds of two sorghum varieties on fermentation of sorghum with lactic acid bacteria for inclusion in poultry diets

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Introduction Wet feeding beneficially affects feed intake and growth rates of poultry in hot climates (Forbes 2003). Fermenting liquid feeds for pigs has been shown to reduce microbial contamination of the feed (Beal *et al.*, 2002) and this could be an added advantage in hot climates. The most desired property of fermented feed is a high lactic acid concentration produced by lactic acid bacteria (LAB) as the fermenting organisms. This high lactic acid concentration resulting in a low pH is responsible for the antimicrobial activity of fermented feeds. This activity is important for food and environmental safety. Grain sorghum is widely used as a food and animal feed cereal in many parts of Africa, Asia and the semi-arid tropics world-wide. It is recognised that differences in the colour of sorghum varieties are due mainly to their concentration of polyphenol compounds mainly tannins. These compounds have some antimicrobial properties and may affect the activity of lactic acid bacteria used to ferment liquid feeds. Little is known about the lactic acid fermentation of sorghum grain for fermented feeds in monogastric nutrition. The present study was designed to compare the fermentation of red and white sorghum with LAB for inclusion as a component of poultry diets.

Materials and methods A study to compare the fermentation of two sorghum varieties (red and white sorghum) with lactic acid bacteria (LAB) was carried out using *Lactobacillus plantarum*, *Lactobacillus farciminis*, *Lactobacillus salivarius*, *Pediococcus acidilactici* and a control treatment without LAB. All cereal samples were hammer-milled and irradiated (25 kGy from Co60) in 50 g sachets before fermentation. There were three replicates per treatment. Feed samples were mixed with water at a ratio of 1 feed:1.4 water then inoculated with 0.1 ml de Man, Regosa, and Sharpe (MRS) broth containing a 24 h culture of one of the four species (providing a concentration of ca 10^6 cfu g^{-1} of feed) and incubated at 30 °C. Samples were collected after 0, 4, 8, and 24 hours of fermentation. The pH was recorded after 24 hour fermentation and samples were taken for analysis of sugar and organic acid concentration by HPLC according to the method of Niven *et al.* (2004). Extraction of phenolic compounds from duplicate samples of red and white sorghum was undertaken using a modified method of the procedure described by Chavan *et al.* 2001. Data on the 24 hour fermentation of the two varieties were compared using the paired t-test while data on the microbial fermentation with time for the two varieties were analysed by the general linear model procedure of analysis of variance using Minitab (release 14.0). Differences between means were determined using Tukey's test. Probability values of $p \leq 0.05$ were considered to be statistically significant.

Results The mean phenolic contents of red and white sorghum were 5.53 ± 0.48 and 4.05 ± 0.80 g/100g grain respectively. The concentration (mM) of maltose, glucose, fructose, lactic and acetic acids after 24 hour fermentation were 2.94, 16.50, 14.03, 266.40 and 10.68 respectively for red sorghum while corresponding values for white sorghum were 3.47, 15.29, 14.67, 273.7 and 11.14. There were no significant differences $P > 0.05$ between the varieties for concentration of sugars and organic acids. Lactic acid concentration was significantly increased by fermentation with all LAB compared with the controls ($P < 0.001$). There were no significant differences in lactic acid concentration after 24 h fermentation with *L. plantarum*, *L. farciminis* or *L. salivarius* regardless of sorghum variety. However, *P. acidilactici* produced significantly less ($P < 0.05$) lactic acid in red sorghum than white sorghum and this was significantly less than the other three organisms. The pH of all fermented sorghum was significantly lower ($P < 0.001$) after 24h than the control, but there were no significant differences in pH after 24 h between fermenting organisms.

Conclusions Sorghum may be successfully fermented for inclusion into wet feed for poultry. Fermentation of sorghum with a range of lactic acid bacteria produce a low pH feed that has a high lactic acid concentration. The results of this study suggest that the *Lactobacillus* spp used were not affected by the phenolic content as there were no significant differences in lactic acid production between red and white sorghum. However, *P. acidilactici* produced significantly more lactic acid in white sorghum which had a lower phenolic content, suggesting that this organism may have been adversely affected by the phenolic content of

Table 1 Lactic acid production and pH after 24h fermentation of red and white sorghum with different lactic acid bacteria

Sorghum	Lactic acid mmol/L		pH	
	Red	White	Red	White
Control	8.2 ¹	12.0 ¹	5.7 ¹	5.1 ¹
<i>L. plantarum</i>	312.3 ^{a1}	313.7 ^{a1}	3.5 ^{a1}	3.2 ^{a1}
<i>P. acidilactici</i>	203.3	264.1 ^a	3.6 ^{a1}	3.4 ^{a1}
<i>L. farciminis</i>	276.3 ^{a1}	252.2 ^{a1}	3.6 ^{a1}	3.3 ^{a1}
<i>L. salivarius</i>	273.8 ^{a1}	265.0 ^{a1}	3.6 ^{a1}	3.3 ^{a1}
s.e.m	10.28	10.28	0.13	0.13

^a no significant difference between means in the same column

¹ no significant difference between means in the same row

red sorghum.

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Effect of split diets on laying performance and egg quality of hens during the late laying period by manipulating the time of access to calcium and phosphorus

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Introduction Nowadays, commercial strains of laying hens lay the majority of their eggs (about 90%) during the morning (Leeson and Summers, 1978). Also, shell is deposited around the albumen when the egg remains in the shell gland. The period of shell deposition coincides mainly in the afternoon and evening. As a result of these processes, hens may have higher calcium (Ca) requirement during the afternoon and in evening. The higher phosphorus (P) requirement in the morning is needed to replace the bone minerals which have been reabsorbed during the previous night when shell formation occurred. (Keshavarz, 1998). Regarding these facts, it has been shown that reducing dietary P during the afternoon and evening had a beneficial effect on shell quality (Roland and Harms, 1976). Hence, we investigated if it would be possible to improve laying performance and egg quality when hens received diets with adequate levels of P only in the morning and Ca only in the afternoon and evening.

Materials and methods There hundred and eighty four (384) 54-wk-old hens of a commercial Leghorn strain (Hi line W-36) were used in a factorial complete random design experiment with four treatments and five replications for a period of seven weeks. Treatments included diets with different available levels of Ca and P in the morning (0500 to 1300 h) and in the afternoon and evening (1300 to 2100 h). Treatment 1 was a control standard diet with 0.25% P and 3.5% Ca levels which were fed in the morning and in the afternoon, equally. Treatment 2 had a lower calcium content (0.5%) in the morning and higher calcium content (6.5 %) in the afternoon and evening, while treatment 3 had a higher P level (0.4 %) in morning and lower P content (0.1 %) in afternoon and evening. In treatment 4, hens received 0.5% Ca and 0.4% available P in the morning, but 6.5% Ca and 0.1% P in the afternoon and evening. Feed intake, egg production (% hen/ days) and egg mass (g hen/ days) were recorded daily. All the eggs produced during three consecutive days (from 1500 h of the first day to 1500 h of the third day) on a bi-weekly period were saved for the measurement of egg weight. 25% of these eggs were used for the measurement of egg components (white, yolk and shell weight), shell thickness, and Haugh unit. In the morning of the last day of experiment one hen from each replicate was killed and the left tibia was saved for ash determination. Data were analyzed for production performance and egg quality in a factorial complete random design by JMP V 4.0.4.

Results The data presented in Table 1 shows that split diets had significant positive effect on feed conversion ratio, cost of feed per egg production, egg mass ($p<0.01$) and egg yield ($p<0.05$), but it had no effect on feed intake, shell thickness, bone ash, Haugh unit, egg weight or components. Treatment 4 had the greatest effects on production performance.

Table 1 Effect of split diets on feed conversion (FC), cost of feed per egg production (CFP), egg mass (EM) and egg yield (EY)

Diet	FC	CFP (Rls)*	EM (g)	EY (%)
1	2.69	5655	42.1	67.5
2	2.48	5221	45.0	71.1
3	2.50	5258	45.1	71.4
4	2.45	5136	46.31	73.2
SE	0.05	98	0.82	1.33
P	<0.01	<0.01	<0.01	<0.05

*1 Rls =0.0011 \$ US

Conclusion This experiment clearly showed that the use of diets with different concentrations of Ca and P in the morning and afternoon/evening, could improve the performance of production without affecting feed intake or egg quality, and hen health during the late laying period.

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Effects of Mintrex® organic trace minerals on growth performance, leg abnormalities and bone breaking strength in turkeys

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Introduction Turkeys can have a high incidence of leg problems and leg-related mortality can exceed 1% per week starting at approximately 15 weeks of age. Development of bone and connective tissue is dependent on a number of factors including the availability of trace minerals. These minerals are essential for the function of the metalloenzymes involved in the synthesis of structural connective tissue and bone. For example Zn and Cu are required for the synthesis and crosslinking of collagen respectively. Vitamin D and its metabolites also play important roles in bone development, as deficiencies can lead to a failure of mineralization, leg weakness and rickets. The aim of the study was to determine the impact of partially replacing inorganic sources of Zn, Cu, Mn and Se in the diet of growing turkeys with an organic trace mineral source, Mintrex®P_{Se}, in the presence or absence of 25-hydroxy D3, on growth performance, leg abnormalities and bone strength.

Materials and methods 864 male Nicholas 85X700 turkeys were randomly assigned among twelve replicate floor pens of 18 turkeys per 9 m² pen. Four experimental treatment groups were randomly assigned among the 4 blocks of 12 pens. The diets for each growth phase were formulated to meet the nutritional needs for eight feed phases based on maize- soybean and fed ad libitum. The four experimental treatments consisted of a 2 X 2 factorial arrangement of two dietary inclusion levels of 25-hydroxy D3 (0 and 92 mcg /kg) and two levels of Mintrex®P_{Se} (0 and 1g/kg). This inclusion level of Mintrex provided 40mg/kg chelated Zn, 40 mg/kg chelated Mn and 20mg/kg chelated Cu (replacing the same amount of inorganic Zn, Mn and Cu as sulphates) and 0.3mg/kg Se as Se yeast (replacing NaSeO₃). The ligand for the Mintrex range of organic trace minerals is the hydroxy analogue of methionine and the contribution of this methionine source was taken into account when balancing the rations for methionine supply. Body weight and feed intake were measured at 3, 6, 8, 10, 12, 15, 17 and 20 weeks of age. Incidence of leg abnormalities (varus, valgus, and shaky leg) and walking ability score (3=down and cripple; 2=resistant mobility with significant limp; 1=mobile with mild limp; 0=no leg-associated mobility problems) was evaluated by observers who were unaware of treatments at 12, 15, 17 and 20 weeks of age. Fracture resistance of 17 week tibias (2 birds per pen) was tested by four-point bending using an axial servo-hydraulic load frame and torsional properties of femurs were tested using an axial-torsion servo-hydraulic load frame (858 Mini Bionix II, MTS Systems Inc., Minneapolis, MN). All data were analyzed as a 2 X 2 factorial design with dietary Mintrex and 25-hydroxy D3 supplementation as main effects. Pen means were used as the experimental unit for most variables except for bones where each individual was considered as the experimental unit. Leg abnormality incidence data were transformed to the arcsine square root before analysis. All variables of statistical significance are based on a probability of P ≤ 0.05. Data were subjected to ANOVA using the GLM procedure of SAS system (SAS Institute, 2003). Means separation was achieved using Tukey's multiple range tests when a significant F statistic was indicated by ANOVA.

Results There was no treatment effect on body weight. The impact of inclusion of Mintrex® organic trace mineral sources and 25-hydroxy D3 on key parameters is shown in the Table. The partial substitution of inorganic trace mineral sources with Mintrex P_{Se} (organic Zn, Cu, Mn and Se) resulted in an improvement in overall feed conversion over the 20 week period, increased tibia strength at 17 weeks, and a reduction in leg problems at all weeks (some data not shown). HyD inclusion sometimes increased leg problems, but also increased bone strength in the presence of Mintrex.

HyD	Mintrex	FCR (20wk)	Total LP ¹ 17wk	Valgus ² 20wk	Tibia Ö _{max} MPa ³	Femur τ _{max} ⁴ (MPa)
-	-	2.78	37.5 ^a	14.1 ^{ab}	112.62 ^b	45.52 ^b
-	+	2.74	5.1 ^b	9.4 ^b	114.48 ^{ab}	41.51 ^b
+	-	2.82	34.7 ^a	24.1 ^a	107.39 ^b	45.32 ^{ab}
+	+	2.70	8.4 ^b	16.9 ^{ab}	129.06 ^a	50.75 ^a
SEM		0.015	3.27	2.87	4.96	2.7
Source of Variation						
Treatment			0.0001	0.0202		
HyD		0.898	0.5283	0.0166	0.3471	0.3001
Mintrex		0.009	0.0001	0.0371	0.0221	0.7844
HyD*Mintrex		0.211	0.1713	0.8506	0.0512	0.0366

^{a,b}Means with different superscripts within a column differ significantly (p<0.05) based on treatment source of variation analysis ¹Total Leg Problems (%all birds with leg problems—varus, valgus or shaky leg) ²Incidence of Valgus at 20 Weeks (% of all birds) ³Maximum normal stress at breakage of tibias in the 4 point assay

⁴Maximum shear stress at failure of femurs in the torsional assay

Conclusions These results support the hypothesis that the Mintrex was more efficient than inorganic trace minerals in delivering essential trace minerals for bone and connective tissue development, thereby reducing leg problems in growing turkeys.

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Comparison on growth of Fayoumi and indigenous chicks reared under semi-scavenging system in two villages of Noakhali district of Bangladesh

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Introduction In Bangladesh about 78% of poultry eggs and 86% of poultry meat are produced by smallholder farmers under scavenging system. The exotic breeds are famous for their high egg production but their adaptability under rural condition is a problem (Islam, 2003). There are reports that Fayoumi chicken, although an exotic breed, can be reared under Asian climatic condition (Bagust, 1994) and therefore may be tried to adapt under our village situation. On the other hand, supplementation of feed is required in order to get optimum growth of the scavenging birds under rural condition. Investigation was done on the growth performance of Fayoumi chicken under semi-scavenging feeding system in rural area of Bangladesh.

Materials and methods The experiment was conducted in two villages of Noakhali district of Bangladesh. Ten farmers, having minimum training on poultry rearing were randomly selected for the study. The experiment was conducted for 12 weeks. Two hundred and sixty day-old chicks (indigenous 130 and Fayoumi breed 130) were divided into two groups based on the type of feed supplementation, prepared feed (30% commercial balanced feed + 50% broken rice + 20% rice polish) and commercial feed (100%). In each of the groups, irrespective of bird type and feed type, there were 5 replications each having 13 indigenous and 13.0 Fayoumi. Each type of birds was fed both prepared and commercial feed as supplementation in addition to scavenging. Same amount of feed (8.0g to 65.0g from 1st to 12th week) was supplied to each bird. Starter and grower diets were supplied from 1 and 28 days and from 29 and 84 days, respectively. The birds scavenged for 8 hours daily. The study was continued for 12 weeks. The parameters measured were live weight gain, FCR and cost profit ratio of birds. Data were analysed according to two- factor (2×2) Completely Randomized Design (CRD) using MSTATC statistical package. The LSDs were used for significant variation.

Results The Fayoumi chicks gained higher weekly live weight than that of the indigenous chicks from 1st to 4th week of the experiment; thereafter the weight gain of indigenous chicks was higher than that of Fayoumi chicks. However, irrespective of the bird type, the chicks fed commercial feed gained higher live weight every week than that of the group fed prepared feed throughout the experimental period. The total mean live weight of indigenous chicks (698.8±31.4g) was significantly (P<0.001) higher than that of the Fayoumi chicks (606.0±22.1g), regardless of feed type. On the other hand, irrespective of breed type, the chicks fed commercial feed achieved significantly (P<0.001) higher live weight (725.6±23.9g) than that achieved by them fed prepared feed (579.2±13.7g). The feed conversion ratio (FCR) of the indigenous chicks (4.7) was significantly (P<0.001) superior than that of Fayoumi chicks (5.5). Similarly, commercial feed gave significantly (P<0.001) better FCR than that of the prepared feed. Survivability of the chicks of either breed or feed type or their interactions was not significantly different between each other. The cost profit ratio was found significantly (P<0.05) higher in indigenous than Fayoumi chicks whereas improved feeding with commercial feed is not cost effective.

Table 1 Performance of indigenous and Fayoumi chicken fed different diets and reared under rural condition

Parameters	Bird type (B)		Feed type (F)		SED	Signf. of contrast [#]		
	Indigenous	Fayoumi	Prepared feed	Commercial feed		B	F	B×F
Initial weight (g/bird)	28.5	28.4	28.2	28.6	0.51	NS	NS	NS
Final weight (g/bird)	698.8	606.0	579.2	725.6	15.94	***	***	NS
Feed supplementation (g/bird)	3108	3108	3108	3108	-	-	-	-
FCR (on supplementary feed, g feed/kg gain)	4.7	5.5	5.7	4.5	0.14	***	***	NS
Survivability (%)	66.4	61.1	59.2	68.3	7.26	NS	NS	NS
Cost profit ratio	3.56	15.01	8.92	9.64	7.52	*	NS	NS

[#] Contrast: B= Main effects between indigenous and Fayoumi. F= Main effects between prepared feed and commercial feed group. B×F= Interaction between main effects

Conclusions It may be concluded that indigenous chicks were better performer in terms of live weight gain, FCR and cost profit ratio than Fayoumi chicks and commercial feed supplementation was better than prepared feed for live weight, live weight gain, FCR of both indigenous and Fayoumi chicks. Therefore, if the scavenging indigenous chicks are supplemented with commercial feed, they will generate more profit than rearing Fayoumi under rural condition of Bangladesh.

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Use of Artemia meal as a protein supplement in broiler diet

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Introduction In spite of superiority of animal proteins as compared with plant proteins, usage of some resources like meat and bone meal maybe restricted, because have salmonella pollution .The other problems that produce by use of fish meal deterioration include, decrease of broiler meat and eggs quality and gizzard erosion. If will not apply suitable temperature in process of fish meal production, thiaminase will remains in supplies and produce dangerous effects. Under condition of feed ingredients deposition before usage by animal, thiaminase will decrease thiamin level. Furthermore existence of trimethylamine in fish meal is caused unpalatable taste and odor in meat and egg that have produced. This situation will more observe when birds unable to produce enough trimethylamine oxidase for elimination of trimethylamine (Klasing , 1998;Leeson&Summers, 2001).Ras *et al.* (2002) in a nutritional experiment, had replacement fish meal by sun cured artemia meal on one-day old broilers during 9 weeks. Diets included fish meal and other contains 2.5, 5, 7.5 and 10 percent of artemia meal. Results showed that mean of broilers weight with 2.5, 5, 7.5 percent of artemia had significant difference in comparison with 10% artemia. There was significant differences between treatments from standpoint of feed intake and feed conversion ratio ($P<0.05$). This researchers had pointed that can use artemia or brine shrimp as a feed ingredients with high level of energy and protein in poultry nutrition (Ras *et al.*, 2002).

Materials and methods Two kind of artemia(*Artemia urmiana*) meal include Urmia Lake Artemia Meal(ULAM) and Earth Pond Artemia Meal(EPAM)was replaced in 5 levels of protein (0,25,50,75,100% of replacing) instead of fish meal proteins and broiler performance was measured.Therefore there were 10 treatments with 3repetitions and each repetition included 10 one-day old broiler male from 308 Ross strain. Feed intake (gm) ,weight gain(gm), feed conversion ratio, carcass traits, abdominal fat, percent relating to weight of small intestine, liver that measured at the end of each week or end of experiment.

Results Effect of kind of artemia and effect of level of replacing it from standpoint of feed intake weren't significant. But there was significant difference for their reciprocal effect ($P<0.05$). The highest feed intake belongs to ULAM (in 50%level) and the lowest depend to without ULAM treatment (5%fish meal). From standpoint of weight gain and feed conversion ratio there wasn't significant difference between treatments. There wasn't significant difference from stand point of carcass traits and GI parts. The length of feeding period was 7 weeks.

Table 1 Comparison of feed intake, weight gain, FCR,carcass yield , abdominal fat ,small intestine and liver (gm or %)

Treatments	feed intake (g)	weight gain (g)	FCR	carcass yield (%)	abdominal fat (%)	small intestine (%)	liver (%)
Kind of artemia :							
ULAM	4542	2126	2.14	61.60	1.24	4.62	2.08
EPAM	4612	2210	2.09	61.80	1.28	4.72	2.10
Level of replacing of fishmeal protein:							
0%	4474	2140	2.10	61.45	1.32	4.66	2.32
25%	4610	2216	2.08	61.71	1.33	4.89	2.14
50%	4739	2151	2.20	61.83	1.36	4.43	2.07
75%	4534	2119	2.14	62.22	1.20	4.86	1.93
100%	4532	2216	2.05	61.27	1.11	4.49	2.08
Kind of Artemia *							
level of replacing of fish meal protein:							
ULAM in level of 0%	4351 ^a	2021	2.16	61.58	1.34	4.33	2.16
ULAM in level of 25%	4435 ^b	2141	2.07	60.88	1.24	4.91	2.14
ULAM in level of 50%	4977 ^a	2190	2.28	62.36	1.27	4.32	2.15
ULAM in level of 75%	4468 ^b	2104	2.12	62.60	1.21	4.85	1.91
ULAM in level of 100%	4481 ^b	2176	2.06	60.57	1.14	4.68	2.05
EPAM in level of 0%	4597 ^{ab}	2258	2.04	61.32	1.29	4.99	2.30
EPAM in level of 25%	4785 ^{ab}	2290	2.09	62.54	1.41	4.88	2.14
EPAM in level of 50%	4497 ^b	2112	2.13	61.30	1.46	4.55	2.00
EPAM in level of 75%	4600 ^{ab}	2134	2.16	61.85	1.19	4.87	1.96
EPAM in level of 100%	4583 ^{ab}	2257	2.03	61.98	1.08	4.29	2.12

Means within the same column with different alphabets differ significantly at ($p<0.05$)

Conclusion From standpoint of feed intake results opposite with outcomes from Ras *et al.*(2002). They didn't observed any significant difference between different level of sun cured Urmia lake artemia meal instead of fish meal for feed intake (Ras *et al.*,2002).Another results were showed that we can replacing of artemia meal instead of fish meal as completely .

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Holo-analysis of the efficacy of acids as pronutrients in pig nutrition

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Introduction Bans on the prescription-free use of pronutrient antibiotics in pig production have stimulated searches for efficient replacements. Previous reviews on the efficacy of acids in pig feeds have been selective. Ravindran & Kornegay (1993) was restricted to 13 weaner tests on citric and eight on fumaric acids and Partanen (2001) excluded acid blends and feeds with antibiotics or copper, in reviewing 46 weaner and 23 finisher tests on formic, fumaric, citric and propionic acids and potassium diformate, but only at dietary levels ≤ 2.5 g/kg feed. This holo-analysis covers acids and salts as acid precursors and their admixtures by elaborating empirical models for feed intake, liveweight gain, feed conversion efficiency and mortality effects in weaners and grower/finishers. The acids studied in the holo-analysis of nutritional response data from 658 negatively-controlled tests are mainly organic, with a minor proportion (5.0%) of inorganic, phosphoric, hydrochloric and silicic.

Materials and methods The total collection of 484 publications (1966-2006) contained 184 with and 141 without performance data, 43 metabolic/mode of action, 37 repeat data, 31 no negative control, 22 no feed intake, eight percentage data, six no acid dosage and 12 miscellaneous. The research encompassed a total of 158 products, including tests on fumaric (103), citric (64), formic (30), propionic (18), phosphoric (18) acids and calcium formate (26), Formi (60), Luprosils (32) and methionine hydroxyl-analogue (MHA) (11), in tests in Germany (33.9%), USA (27.2%), Denmark (9.0%), Canada (6.8%) and 21 other countries (23.1%). The data bank contains 658 (468 weaner and 165 grower-finisher) 13-137 day tests versus 316 negative controls, using 37,924 pigs (mean 57.6/test). Holo-analysis (Rosen, 2006) elaborates empirical multiple regression models quantifying dependent variable responses in terms of all available genetic, environmental, managemental, dietary and nutrient independent variables. It deploys standard JMP[®] (2000) multiple regression methodology for best-fit response models, with maximum multiple regression coefficient squares (R^2) and minimum root mean square errors (RMSE), using a conventional $P \leq 0.05$ in/ $P \geq 0.10$ out probability criterion excluding aberrant $\geq 3 \times$ RMSE residual responses, for 43 independent variables, including linear, quadratic or logarithmic feed dosages (g/kg).

Results The models for the effects of acids, precursor salts and admixtures on feed intake, liveweight gain and feed conversion ratio contain a total of 23 significant independent variables, plus three product terms (dosage x control performance, dosage square x control performance and duration x control performance), accounting for 40-48% of variations in response. The feed intake model contains significant positive terms for dosage square, control feed intake, duration, year of test, dosage x control feed intake, part-purified diet, antibiotic feed, non-cereal carbohydrate (%), animal protein (%), disease, Formi and MHA and negative terms for dosage, dosage square x control feed intake, control feed intake x duration and vegetable protein content (%). The liveweight gain effect model contains positive terms in dosage square, duration, year of test, dosage x control liveweight gain, part-purified diet, antibiotic feed, non-cereal carbohydrate (%), cage/wire floor, Formi, MHA and phosphoric acid and negative terms in dosage, control liveweight gain, dosage square x control liveweight gain, duration x control liveweight gain, inorganic acid, vegetable protein content (%), main cereal content (%) and pure-bred pig. For the feed conversion ratio model there are adverse (positive) terms for duration, dosage x control conversion performance, inorganic acid, straw/shavings bedding, main cereal content (%), USA test, mash feed and pure-bred pig with negative (beneficial) terms for dosage, dosage square, control feed conversion ratio, part-purified diet, antibiotic feed, cage/wire floor, pelleted feed and MHA. The mortality effect model based on 29 tests (R^2 0.968) contains negative terms in control mortality (%), non-cereal carbohydrate (%) and individually-housed/fed pig with a positive term for USA test. Using the aforementioned feed and gain models to compare response expectations in praxis in apparently-healthy pigs fed practical diets at today's levels of performance with the mean responses observed in the research over 40 years, the mean effect on feed conversion improvement of acids is -0.041 compared with the research average of -0.073 . Whilst the former is less than that under the research conditions, it is nonetheless a sound indicator of the potential for acids in pig production as a contributor to antibiotic replacement in pig feeds.

Conclusion As yet the Acipig project has yielded no clear-cut indication of optimal dosages for acids in pig nutrition. More dose-response studies are required testing 0, 5, 10, 20, 30, 40 and 50g/kg, excluding the use of impractical part-purified diets and non-cereal carbohydrate ingredients, except lactose, manioc and molasses. Much benefit from further research would accrue if authors were to routinely provide key data often missing from their reports, e.g. full diet composition, temperature, altitude, etc. in order to elucidate the significant biological factors underlying the significant unexplained influences of geographic (USA) and temporal (year of test) effects reported herein.

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Dietary betaine and ractopamine have additive effects on lean tissue deposition in restrictively-fed gilts

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Introduction Dietary ractopamine supplementation dramatically increases the rate of carcass lean deposition, with the lean tissue responses most evident at *ad libitum* feed intakes (Dunshea *et al.* 1998). Dietary betaine can improve growth by reducing maintenance requirements, with the greatest responses generally occurring when DE is limiting. For example, dietary betaine stimulated lean tissue deposition in boars consuming 80% but not 100% of *ad libitum* (Suster *et al.* 2004). Importantly, the effects of betaine were additive with effects of porcine somatotropin in the restrictively-fed boars. Therefore, the aim of the present study was to investigate the interactions between dietary ractopamine and betaine on growth performance and carcass characteristics in restrictively-fed finisher pigs.

Materials and methods Forty individually penned pigs (initial weight 58.4 kg) were allocated to a 2 x 2 x 2 factorial experiment with the respective factors being sex (gilt or boar), dietary betaine (0 and 1.25 g/kg Betafin, B) and dietary ractopamine (0 and 10 ppm Paylean, RAC). Pigs were weighed weekly and feed offered for the next week was adjusted based on their live weight. Pigs were offered 80% of *ad libitum* for the first 14 days and 88% of *ad libitum* for the next 20 days of the study. *Ad libitum* feed intake was estimated to be 125 g/kg^{0.75} for our genotype. Average energy intake over the entire study was 34.5 MJ DE/d which is similar to that observed on commercial farms (Dunshea 2005). Diets contained 72% wheat and 16% canola meal and were formulated to 0.56 g available lysine/MJ DE (1.0% total lysine) and 13.8 MJ DE/kg. Back fat thickness at the P2 site (6.5 cm from the midline over the last rib) was determined at day 0 and day 34. Body composition was determined by dual energy X-ray absorptiometry at 0 and 35 days (Suster *et al.* 2003).

Results Over the first 14 days of the study daily gain was increased in pigs fed betaine (+8%, $P=0.04$), was greater in boars (+12%, $P=0.005$) but was not effected by RAC ($P=0.18$). Over the same period, there were similar improvements in feed efficiency. However, growth and efficiency responses were not maintained over the entire study. Lean deposition tended to be greater in pigs fed betaine (+5%, $P=0.08$), was greater in boars (+6%, $P=0.006$) but was not effected by RAC ($P=0.57$). However, there was an interaction ($P=0.03$) between RAC and sex such that RAC increased lean deposition in gilts but not boars. As a result betaine and RAC had additive effects on lean mass in gilts (+5.1 kg) but not boars (Figure 1). Fat deposition was less in pigs fed RAC (-8%, $P=0.05$), was lower in boars (-17%, $P<0.001$) but was not effected by betaine ($P=0.81$). However, there was an interaction ($P=0.04$) between dietary RAC and sex such that RAC decreased fat deposition in gilts (-14%) but not in boars.

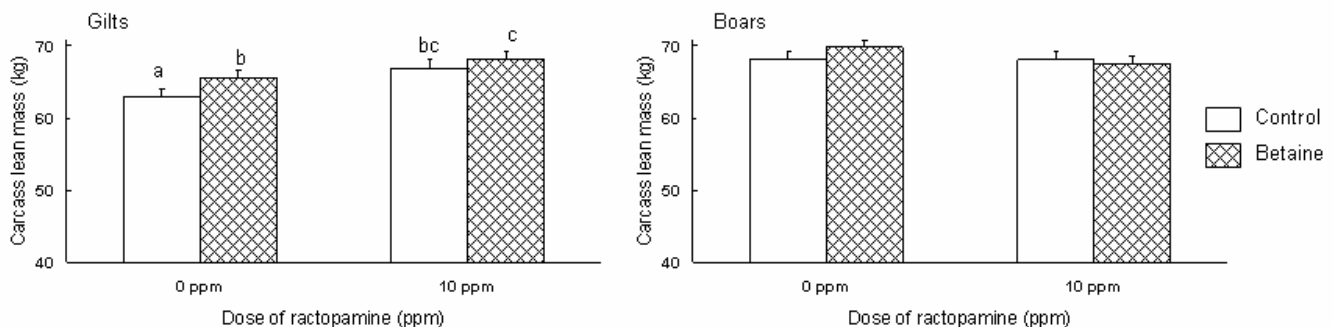


Figure 1 Effect of dietary betaine and ractopamine on lean tissue mass as slaughter in gilts and boars

Conclusion Dietary betaine improved growth rate and lean tissue deposition, consequently increasing the lean tissue content of the carcass at slaughter in restrictively fed animals and these effects were additive with those of ractopamine. However, these responses appeared to be most pronounced in gilts rather than boars. These data demonstrate that when energy intake is limiting the potential for growth, as is normally the case for the improved gilt, combined dietary betaine and ractopamine treatment can increase lean tissue deposition and decrease fat deposition.

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Effects of lactose inclusion level and inulin inclusion on performance of newly weaned pigs a large-scale facility

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Introduction Feeding non-digestible, fermentable carbohydrates that do not lead to increased viscosity may reduce risk of enteric disorders arising from feeding high protein diets to newly weaned pigs (Wellock *et al.* 2007). Lactose and inulin are such fermentable carbohydrates. Lactose is fermented at a relatively high rate, and would thus affect gut environment in the distal ileum and proximal large intestine. In contrast, inulin is fermented more slowly and would be expected to affect gut environment in the proximal and distal large intestine. Consequently, their combined use could be expected to affect gut environment throughout the distal gastrointestinal tract, and through their prebiotic action reduce risk of enteric disorders (Wellock *et al.* 2007). The objective of the current experiment was to investigate the combined effects of lactose level and inulin inclusion on the performance and health of weaned pigs in the immediate post weaning period, in the absence of anti-microbial growth promoters, therapeutic levels of ZnO and CuSO₄ in a commercial, large-scale facility.

Materials and methods A 2 x 2 factorial design experiment compared dietary lactose level (H, 200 g/kg vs L, 100 g/kg) and inulin inclusion (+, 20 g/kg vs - not included) over five experimental rounds. Diets contained 16.0MJ DE, 220 g CP and 16 g lysine per kg. Three hundred and forty (JSR Gold x Hampshire) pigs of mixed sex were weaned at 26 ± 2.4 (S.D.) days of age and 8.6 ± 1.1 kg body weight, balanced for initial weight and litter and offered *ad libitum* access to one of the four dietary treatments for 14 days post weaning. The main ingredients of the basal diet (g/kg) were porridge oats (90), micronised cereals (244), full fat soya (150), Hamlet protein, a commercial soy-protein source (50), fishmeal (75), delactosed whey (130) and whey protein concentrates (62). Extra lactose and inulin were included at the iso-energetic expense of a mixture of wheat starch and soya oil. From day 14 pigs were fed standard commercial diets until the end of the weaner phase at approximately 30kg body weight (days 14+). Pigs were housed in groups of 6-8. Individual weight gain, pen feed intake and faecal score (assessed through subjective scoring from 1 to 4, with increasing degree of fluidity) were recorded. Fresh faecal samples were collected on day 11 post weaning for enumeration of lactobacilli and coliform populations and to assess enterotoxigenic *E. coli* (ETEC) shedding. Data were analysed by ANOVA with pen as experimental unit, experimental round as fixed effect and mean pig weaning weight as covariate.

Results Table 1 shows the effect of lactose level and inulin inclusion on pig performance and health. Throughout the trial period, neither lactose level nor inulin inclusion significantly affected pig performance. Pigs fed high lactose diets tended to have a higher faecal score (i.e. more liquid faeces) but observed differences were small. In addition, pigs fed inulin-enriched diets tended to excrete lower numbers of ETEC but overall ETEC excretion was low. Lastly, feeding treatment did not affect the faecal lactobacilli to coliform ratio.

Table 1 The effect of lactose level (L vs H) and inulin inclusion (- vs +) on pig performance, ETEC shedding and lactobacilli to coliform (L:C) ratio throughout the trial period

	Feeding treatments				SED	P values		
	L-	L+	H-	H+		Lactose	Inulin	L x I
ADG days 0-14 (g/d)	200	196	195	174	17.0	0.262	0.307	0.492
ADG days 14+ (g/d)	527	522	528	543	23.0	0.486	0.670	0.560
ADFI days 0-14 (g/d)	274	281	274	272	15.0	0.621	0.791	0.659
ADFI days 14+ (g/d)	792	766	765	738	26.0	0.145	0.154	0.986
FCR days 0-14	1.52	1.54	1.48	1.70	0.15	0.592	0.262	0.349
FCR days 14+	1.52	1.48	1.48	1.39	0.07	0.224	0.232	0.670
Faecal score (1-4 scale)	2.69	2.60	2.73	2.82	0.11	0.098	0.914	0.220
ETEC (log ₁₀ cfu/g)	0.50	0.00	1.49	0.39	0.68	0.305	0.095	0.425
L:C ratio	1.37	1.37	1.31	1.27	0.06	0.210	0.498	0.968

Conclusion Under the conditions of the trial, lactose and inulin did not affect pig performance and gut health, as indicated by the similar L:C ratio. In addition, although the relatively high mean faecal score would suggest that pigs were at risk for intestinal disorders (Wellock *et al.* 2006), the low level of ETEC excretion observed indicates that infection pressure was probably low. Effects of dietary lactose and inulin on performance and gut health of newly weaned pigs in more challenging nutritional and/or infectious environments remain to be assessed.

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Effect of lactation environment and dietary zinc oxide level on the response of group housed weaned pigs to enterotoxigenic *Escherichia coli* (ETEC) O149 challenge

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Introduction Proliferation of ETEC in the small intestine is recognised as the predominant cause of post-weaning colibacillosis (Marquardt *et al.*, 1999). Established ETEC secrete toxins that disrupt normal enterocyte function and lead to diarrhoea, dehydration, poor performance and increased mortality. Zinc oxide (ZnO) addition to the diet increases small intestine mucosal growth and promotes normal intestinal function. In turn the incidence and/or severity of diarrhoea is reduced and performance is improved (Li *et al.*, 2001, Ragland *et al.*, 2006). Pre-weaning environment also influences performance with outdoor reared pigs growing faster to weaning than indoor reared contemporaries (Miller *et al.*, 2007) but are outdoor reared pigs able to cope with ETEC challenge at weaning? The current experiment compared the responses of group-housed pigs weaned from different lactation environments and fed diets differing in ZnO content to a prescribed ETEC challenge.

Materials and methods The experimental design and all procedures were approved by the Ethical Review Committee of the University of Leeds and were carried out under Home Office licence. The experiment consisted of a 2 x 2 factorial combination of rearing environment (indoors [In] vs outdoors [Out]) and two levels of dietary ZnO supplementation (zero [-Zn] and 3.1g/kg of diet [+Zn]). All pigs were given access to creep feed without ZnO from 14 days pre-weaning. Pigs were weaned at 27.5 days of age \pm 0.24 (\pm sem) and 7.35 \pm 0.16 kg live weight. Two male and two female pigs (Large White x Landrace) were selected from each of eight In and eight Out litters to generate eight pens of four pigs from each rearing environment. Within environment pens were balanced for litter; weaning weight and sex were balanced across all pens. The two trial diets (210g CP, 16.5MJ DE and 16.4g total lysine per kg; with and without ZnO) were allocated evenly across pens within environment. Diets were provided ad libitum from multispace feeders; water was freely available throughout. Feed intake and piglet liveweight were recorded daily for the duration of the trial. On day three post-weaning pigs were challenged *per os* with 10⁹ cfu of ETEC O149. Faecal ETEC counts were determined at weaning, immediately prior to challenge and daily thereafter for five days. On day seven post infection all pigs were killed for measurement of tissue responses and lactobacilli:coliform ratio. Data were analysed using the GLM procedures of Minitab 12.2 with experimental units of pen for the performance measures and individual pig for the tissue and microbiological data.

Results ETEC excretion was significantly reduced in Out and +Zn pigs (P<0.01); furthermore these effects were additive (Table 1). Outdoor rearing increased small intestine (SI) and colon weight (P<0.05) but had no significant effect on intestinal morphology or pig performance. Addition of ZnO to the diet enhanced villus height and villus goblet cell numbers in the upper SI (P<0.05), increased lactobacilli to coliform ratios (L:C) in the lower SI and proximal colon (P<0.05) and improved feed intake, growth rate and feed conversion ratio (P<0.05). Moreover, beneficial effects of outdoor rearing on upper SI villus height and pig performance were dependent on the addition of ZnO to the diet.

Table 1 Treatment effects on intestinal structure, microbiology and performance of pigs in response to ETEC infection

	In -Zn	Out -Zn	In +Zn	Out +Zn	sem	Significance		
						Env.	Diet	Env*Diet
Upper SI villus height (μ m)	307	267	302	346	16.0	-	P<0.05	P<0.05
Upper SI villus goblet cells/100 μ m	1.6	2.1	2.5	2.8	0.28	-	P<0.05	-
Mean faecal ETEC (log ¹⁰ cfu/g)	6.8	5.9	5.7	4.6	0.38	P<0.01	P<0.01	-
Terminal ileum L:C	1.14	1.20	1.26	1.40	0.072	-	P<0.05	-
Proximal colon L:C	1.10	1.15	1.16	1.25	0.040	P<0.1	P<0.05	-
Feed intake (g/pig/day)	238	222	252	305	21.4	-	P=0.05	P<0.1
Daily gain (g/pig/day)	233	174	277	347	27.2	-	P<0.01	P<0.05
Feed conversion ratio	1.02	1.44	0.91	0.84	0.144	-	P<0.05	-

Conclusion These results suggest different but complementary mechanisms of counteracting ETEC consequences in outdoor reared pigs and those offered ZnO supplemented diets. However, the data clearly indicate that the benefits of ZnO addition to the diet extend beyond suppression of ETEC and may be mediated through altered development of the SI mucosa.

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The effect of offering two feeds, with or without pharmacological levels of zinc oxide, on the individual feeding behaviour and performance of weaned piglets

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Introduction Zinc oxide (ZnO) is frequently added to nursery piglet diets at pharmacological levels to combat scouring and to improve performance of weaned piglets (Miller and Slade, 2006). It is anecdotally recognized in the pig industry that piglets do not like the taste of zinc although increased feed intake is frequently reported (Ragland *et al.*, 2006). Dietary selection studies have demonstrated the ability of the pig to discriminate between different foods (Forbes and Kyriazakis, 1995). This experiment aimed to test the hypothesis that weaned piglets would avoid a diet high in ZnO when given the choice, and to investigate the resultant performance and choice feeding behaviour of individual piglets in the immediate post weaning period.

Materials and methods Sixty piglets (Large White x Landrace) were weaned at $7.8 \text{ kg} \pm 0.14$ (s.e.m.) and 27.8 ± 0.11 days of age into eight mixed sex groups of 7 or 8 piglets per pen. Groups were balanced for litter origin, weaning weight and sex, and housed in fully slatted weaner pens (1.7 m^2). Piglet feeding behaviour was constantly recorded by a multi-spaced feed intake recording system (Leeds University Pig Intake System (LUPINS)) in each pen. Piglets were identified by LUPINS using an individual transponder ear tag. Each pen of pigs was offered *ad-libitum* access to two different foods, each in two troughs per pen. Feeding time (minutes) was recorded for each individual at each trough. Foods (16.2 MJ DE , 1.6 g lysine /kg) differed only in the level of ZnO supplementation: unsupplemented (U) or supplemented (Z: 3.1 g/kg). Food U contained a basal level of zinc (100 ppm). Piglets were weighed at weaning and at day 7 and 13 thereafter. The experiment ran for 13 days. Piglets were categorised by weaning weight into three categories: S (Small $\leq 7.0 \text{ kg}$), M (Medium $\geq 7.1 \text{ kg} \leq 8.0 \text{ kg}$) and L (Large $\geq 8.1 \text{ kg}$). Any piglet observed with post weaning scour was recorded. All data were analysed using the GLM procedures in Minitab 14. Individual data were analysed with piglet as the experimental unit, and for analysis of feed intake and performance, pen was the experimental unit.

Results On a pen basis, groups of piglets showed significant preference ($p < 0.001$) for the unsupplemented food in both week 1 and 2 post weaning (Figure 1). Average piglet gain was low, at 0.039 ± 0.03 and $0.272 \pm 0.04 \text{ kg/day}$ in week 1 and 2 respectively. When feeding behaviour data were examined on an individual basis, the effect of weaning weight and gender on proportion of time spent eating each food did not influence diet choice (table 1). Scouring piglets did not show any increased preference for zinc oxide.

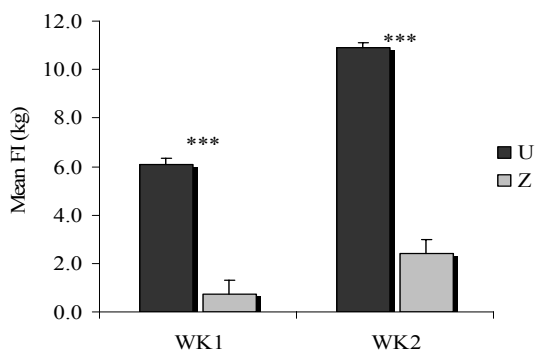


Figure 1 Weekly pen feed intake per food (kg)

Table 1 Proportion of time spent eating each food

	Week 1		Week 2	
	U	Z	U	Z
Wean weight (kg)				
≤ 7.0	S 0.79	0.21	0.79	0.21
$\geq 7.1 \leq 8.0$	M 0.81	0.19	0.74	0.26
≥ 8.1	L 0.86	0.04	0.76	0.24
	sem 0.04	0.04	0.04	0.04
	p - value ns	ns	ns	ns
Sex				
Female	0.84	0.16	0.77	0.23
Male	0.80	0.20	0.76	0.24
	sem 0.03	0.03	0.03	0.03
	p - value ns	ns	ns	ns

Conclusion Weaner piglets strongly preferred feed without ZnO inclusion and it is clear from our results that the palatability of the feed was reduced by ZnO. None the less, a nominal amount was still eaten, suggesting the piglets consumed a small amount in 'continuous sampling behaviour' (Forbes, 1995), albeit insufficient to benefit the piglet. ZnO has proven positive effects on piglet health and performance post weaning, although the newly weaned piglet would have no prior experience of such. Indeed, the proportion of time spent eating food Z was not influenced by weaning weight or gender. Ironically, diet selection by piglets on this experiment is associated with poor performance and incidence of scour, highlighting the importance of ZnO on the health and performance of the weaned animal.

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Intestinal gene expression in response to zinc oxide and rearing environment in the ETEC-challenged weaned piglet

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Introduction Enterotoxigenic *Escherichia coli* (ETEC) K88 is a common cause of post-weaning scours in piglets. Supplementation of the post-weaning diet with pharmacological levels of zinc oxide reduces the incidence and severity of scours (Carlson *et al* 2004), in particular that caused by ETEC K88 (Owusu-Asiedu *et al.* 2003). Piglets reared outdoors pre-weaning also perform better post-weaning than those reared indoors, with an increased growth rate (Miller *et al.*, 2007). The objective of this study was to identify genes that are differentially expressed in the small intestine of the ETEC-challenged piglet in response to dietary ZnO supplementation and outdoor rearing, to increase understanding of the mechanism by which these factors exert their effects.

Methods The experiment consisted of a 2 x 2 factorial design, as detailed in Slade *et al* (BSAS proceedings 2008). 64 Large White x Landrace piglets were reared either indoors or outdoors and weaned at 28d onto diets containing either basal zinc (0.1g/kg) or supplemented zinc oxide (3.1g/kg). Three days post-weaning all piglets were orally infected with 10⁹ E.coli (O149:K91:K88), and seven days post-infection piglets were killed and sampled. Mucosal and Peyer's patch tissue scrapings were collected from which total RNA was extracted. A subset of six samples were pooled from each treatment (In basal Zn, In ZnO, Out basal Zn, Out ZnO) and tissue type and hybridised to separate Affymetrix Porcine GeneChip[®] Microarrays. Pairwise comparisons were used to determine differentially expressed genes (two-fold or greater) according to treatment. Primers were designed for the genes identified, and quantitative real-time PCR (QPCR) was performed for each gene on all samples. Ct values were normalised using three housekeeping genes and delta Cts were analysed using the GLM procedure on Minitab 14. Mean expression ratios were calculated using the mean delta Cts for each treatment.

Results Piglet performance data is presented elsewhere (Slade *et al.*, 2008). From a total of 23,937 porcine probe sets on the Affymetrix Porcine GeneChip, 20 genes were identified as differentially expressed in ZnO-treated piglets compared to their basal Zn-fed contemporaries, and eight in outdoor-reared piglets. From these genes, fourteen (mostly involved in the innate immune response) were identified as of particular interest and primers were designed for QPCR. Of these, the genes identified as differentially expressed according to rearing environment were not validated using QPCR, but ZnO-treatment (table 1) resulted in down-regulation in several innate immune response genes in one or both tissues, and up-regulation of the metallothionein genes.

Table 1 Mean fold change in expression of genes of interest using real-time PCR

Gene Name	Epithelia		Peyer's Patch	
	Mean fold change	P value	Mean fold change	P value
Metallothionein-IE	+217	<0.001	+352	<0.001
Elafin precursor	-12.5	<0.001	-15.6	<0.001
Dual oxidase 2 precursor	-7.1	<0.001	-7.7	<0.001
Macrophage inflammatory protein-2-alpha	-3.1	<0.001	-3.2	<0.001
Cytokine B13 precursor (CXCL13)	-3.5	<0.01	+1.1	0.498
Cytokine B6 precursor (CXCL6)	-2.2	<0.001	-2.5	<0.001
Bactericidal permeability-increasing protein	-3.0	<0.01	-3.1	<0.001
Secreted phosphoprotein I	-2.0	0.050	-2.1	<0.001
Pulmonary surfactant-associated protein D	-2.4	<0.01	-2.7	<0.001

Conclusions No genes were identified as differentially expressed between pre-weaning rearing environments, probably due to the 10d period between weaning and sampling. However, QPCR analysis has validated several genes identified as differentially expressed between post-weaning dietary treatments using the Affymetrix GeneChip. The metallothionein family (involved in regulation of Zn absorption) were significantly up-regulated in response to ZnO, in support of previous work. Several innate immune response genes are down-regulated, including elafin, dual oxidase 2 and bactericidal permeability-increasing protein, which have been found to be up-regulated in human inflammatory bowel disease patients. This indicates a reduction in the local innate immune response with ZnO treatment, although it remains to be determined whether altered expression of these genes is a result of a change in intestinal bacterial populations and hence a change in immune response, or whether the mucosal innate immune response is itself controlled to some extent by ZnO.

Acknowledgements This work was funded by BBSRC, ABNA, DSM, Primary Diets and British Quality Pigs.

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The effect of straw supplementation on the performance, physiological development, aggression and boar taint compounds in pigs slaughtered at 120kg live weight.

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Introduction In the UK, markets for ‘welfare friendly’ meat have increased the use of more extensive, deep-bedded straw housing. Straw yard housing may increase physiological development at slaughter (Salmon & Edwards, 2007), with many confounded potential causal factors including increased space allowance, natural lighting/photoperiod, straw availability, increased activity levels and reduced social stress. Physiological development at slaughter is a key issue in the success of heavy pig production utilising entire boars, since greater maturity may increase adverse behaviours, therefore decreasing pig welfare, and increase risk of carcass taint. The objectives of this research were to investigate whether straw supplementation, in the absence of confounded space and lighting effects, influenced physiological development and associated undesirable effects in entire boars and gilts grown from 60kg to 120kg slaughter weight.

Materials and methods 96 Large White x Landrace pigs were randomly allocated at 63kg live weight (LWT), in pens of 8 (4 boars, 4 gilts). They were housed in controlled-environment housing with un-bedded part-slatted pens, provided with a plastic toy for enrichment. Straw treatment pens (STY), were provided with free access to long wheat straw held in a wall-mounted rack (60 x 45 x 20cm). Pigs were fed a commercial pelleted finisher diet *ad-libitum*, with pig live weight and pen feed intake recorded fortnightly from 63kg to 100kg and weekly from 100kg to slaughter at 120kg LWT. Skin lesion scores were recorded fortnightly and rindside damage post slaughter. For an assessment of maturity, ovary weights and follicle scores or testicle weights were recorded at slaughter. Pen mean values were subject to analysis of variance to compare treatment effects and interactions on performance, physiological maturity and related measures.

Results Mean daily straw usage was 58g/pig. Over this heavy weight range, boars showed higher daily live weight gain (DLWG), compared with gilts (P=0.009). There were no effects of STY on daily feed intake (DFI), but DLWG tended (P=0.066), and feed conversion ratio (FCR) (P=0.012), was improved. Carcass back fat depth was greater in boars (P=0.038), and tended to be reduced in STY (P=0.051). There was no effect of STY on any boar or gilt physiological maturity parameter measured (data not presented), and, whilst boars showed significantly greater fat concentrations of skatole (P<0.001), STY exerted no effect on fat androstenone (P=0.698), or skatole (P=0.536), concentration. Adrenal gland weight was greater in boars than gilts (P=0.048), but unaffected by STY (P=0.760). Boars exhibited significantly greater total body lesions (P<0.001), and rindside damage (P=0.031), compared with gilts, but neither measure was affected by STY (P=0.838 and P=0.252). STY treatment showed increased lesions on the rump and tail area only (P=0.015 and P=0.019), but showed only a numeric increase in activity level (P=0.168), and a tendency to increase biting interactions (P=0.086). STY decreased nose-nose interactions (P=0.006), but showed only a tendency to reduce nose-body interaction (P=0.051).

Table 1 Effects of gender (G), and straw provision (S) on performance, stress, taint and aggression measures

	Control		Straw		SEM	Sig
	Boar	Gilt	Boar	Gilt		
DFI (kg)	2.53		2.57		0.069	NS
FCR	3.02		2.77		0.048	S*
DLWG (kg)	0.87	0.77	0.94	0.83	0.033	G**
Carcass back fat (mm P ₂)	13.6	13.5	13.8	11.6	0.49	G*, S*
Fat androstenone (µg/g) ^b	0.73	ND	0.75	ND	0.045	NS
Fat skatole (µg/g) ^b	0.19	0.02	0.15	0.02	0.03	G*** ^a
Paired adrenal gland weight (g)	2.66	1.64	2.19	1.95	0.243	G*
Rindside damage score (1-5)	2.2	1.5	1.7	1.6	0.17	G**
Total body lesion (no./pig)	1.5 (27.2)	1.3 (20.7)	1.5 (29.1)	1.3 (20.2)	0.036	G***
Rump lesion (no./pig)	0.4 (1.6)	0.4 (1.5)	0.5 (2.3)	0.5 (2.1)	0.036	S*
Tail lesion (no./pig)	0.2 (0.5)	0.2 (0.5)	0.2 (0.6)	0.2 (0.7)	0.021	S*
Activity level	0.26		0.28		0.010	NS
Biting	0.10		0.13		0.013	NS
Nose-body	0.98		0.75		0.076	NS
Nose-nose	0.37		0.26		0.025	S**

log transformed for analysis (back transformed means in brackets) ^askewed; non-parametric analyses ^bindividual pig data

Conclusion STY improved growth efficiency but did not influence physiological development at slaughter or carcass taint. 7% of boars had taint levels above thresholds considered detrimental to consumer acceptance. STY improved welfare by reducing pig directed behaviour, but this was not reflected in reduced aggression or lesions.

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Effects of prenatal undernutrition on lamb growth and gastrointestinal parasitism in two breeds of sheep

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Introduction Prenatal undernutrition can have a permanent ‘programming’ effect on offspring growth, health, behaviour and reproductive ability that persists into adulthood (Meaney *et al.*, 2007; Bell, 2006). However, whether different genotypes are equally affected is unknown. Relatively slower growing breeds of sheep, that are adapted to poorer food availability in pregnancy (such as the Scottish Blackface) may be better able to buffer their developing foetuses from the effects of undernutrition *in utero* than faster growing breeds, such as the Suffolk. In this experiment we investigated whether a 25% reduction in food intake for the first 90 days of gestation would result in differential responses in these two breeds of sheep.

Materials and methods One hundred pregnant ewes were assigned to either control (C: 100% requirements for maintenance and foetal growth: n=22 Blackface, 25 Suffolk) or restricted diets (R: 75% requirements: n=28 Blackface, 25 Suffolk) on day 1 following artificial insemination with semen from one ram per breed. Ewes were housed on sawdust and fed an appropriate daily allowance of concentrates to achieve C and R, and offered straw as a forage. Ewes were scanned for litter size on day 50 of gestation and their nutrition was adjusted accordingly. On day 91 of pregnancy, all ewes were given straw bedding, and fed hay and concentrates to meet requirements. These ewes gave birth to 153 lambs (65 Blackface: 36 singles, 26 twins, 3 triplets; 88 Suffolk: 20 singles, 46 twins, 18 triplets, 4 quadruplets) and were moved, with their lambs, to pasture at three days after birth. Multiple litters were reduced to two lambs and non-mother raised lambs are not included in analysis. Lambs were weighed and had crown-rump length (CRL) measures taken at birth, and then weighed at monthly intervals until weaning at 4 months. For three sets of twin litters for each breed treatment combination, faecal samples were collected at weaning for the assessment of strongyle egg counts. The effects of birth litter size, lamb gender, breed and treatment were investigated using the Restricted Maximum Likelihood procedure in GenStat 8, fitting ewe identity as a random factor. Faecal egg count data were square root transformed before analysis.

Results Lamb birth weight and CRL were significantly affected by litter size ($P<0.001$) and breed (weight: $P<0.001$; CRL: $P=0.024$), but there was no overall effect of treatment. However, there was a significant interaction between breed and treatment with a decrease in birth weight in R Suffolk lambs that was not seen in Blackface lambs (Table 1, $P=0.003$). R Suffolk lambs were also significantly shorter than C Suffolk lambs, but there was no effect of undernutrition on CRL in Blackface lambs (Table 1, $P<0.001$). There were significant interactions between time and breed ($P=0.005$), and time and treatment ($P=0.006$) in lamb weight change to 5 months, such that the effects of nutritional treatment on weight had disappeared by weaning (Table 1). There were no overall breed or treatment effects on strongyle ova in faeces collected at weaning. There was, however, a significant breed by treatment interaction (Table 1) with R Suffolk lambs having significantly higher worm burdens than other groups ($P=0.033$). Ova from other worm species occurred at low frequency and were not significantly affected by breed or treatment.

Table 1 Lamb birth and weaning weights, and strongyle egg counts in control (C) and restricted-fed (R) ewes

Variable	C Blackface	R Blackface	C Suffolk	R Suffolk	s.e.d.	Effects
Birth weight (kg)	2.97	3.18	4.63	4.08	0.18	Breed*treat: $P=0.003$
CRL (cm)	41.41	43.17	45.03	42.34	0.79	Breed*treat: $P<0.001$
Weaning weight (kg)	25.93	26.93	29.37	29.66	1.34	Breed: $P=0.001$
Strongyle egg count (epg)	162.6 (90 – 246.6)	110.0 (52.1 – 189.3)	155.8 (84.8 – 238.1)	306.6 (202.8 – 432)		Breed*treat: $P=0.033$

Discussion These data demonstrate that significant genotype differences exist in the ability of ewes to buffer their foetuses from the effects of prenatal undernutrition. In the Suffolk breed, despite adequate nutrition for the last trimester of pregnancy when foetal growth is maximal, R lambs were significantly smaller and shorter than C lambs, whereas the weight and length of Blackface lambs were not affected by undernutrition in the first two trimesters. Similarly, a breed by treatment interaction was seen in the effects of early life undernutrition in the ability of lambs to respond to gastrointestinal parasites, which may have been reduced in R Suffolk lambs. The latter may be associated with displaying some degree of compensatory growth, as maternal under nutrition in early gestation did not affect weaning weight in either breed. The data suggest there may be a programming effect of early life undernutrition, and that this has a greater effect in fast growing breeds which may be less well adapted to low food availability in pregnancy.

Acknowledgements

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The effect of season of shearing on the periparturient rise in nematode egg output in ewes

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Introduction Previous studies (Keady *et al.*, 2007; Keady & Hanrahan, 2007) at Athenry have shown that shearing ewes at housing increases subsequent lamb birth and weaning weights. Keady *et al.*, (2007) reported that shearing at housing increased gestation length whilst Keady and Hanrahan (2007) reported that shearing increased food intake, both indicative of reduced heat stress. The temporary loss of acquired immunity to gastrointestinal nematodes that is associated with an increased faecal egg output in late pregnancy and early lactation, is a well established phenomenon and is important in the epidemiology of nematode parasites of sheep. Given the association between nutrition (Donaldson *et al.*, 1997; 1998; Coop and Kyriazakis, 1999; Houdijk *et al.*, 2000) and the periparturient rise in nematode eggs (PPR) in ewes, the increased food intake evoked by shearing at housing, may have an impact on the PPR. The aim of this study was to evaluate the effect of season of shearing on the periparturient rise in nematode egg output in ewes.

Materials and methods One hundred and thirty ewes (66 first crop, 64 second crop) were allocated at random to one of four shearing treatments namely; conventional (shorn 29 May), conventional & pre-mating (shorn 29 May and 9 September), pre-mating (shorn 9 September), and housing (shorn 30 November). Ewes were managed as one flock, had not been administered an anthelmintic in the previous year and, for mating were synchronised using progesterone impregnated sponges. The ewes were housed (slatted pens) in mid December and offered silage based diets and supplemented with a total of 21kg concentrate during the last 6 weeks of pregnancy. The ewes were turned out to pasture within 3 days of lambing. Triplet rearing ewes were offered 1kg of concentrate daily for 5 weeks post lambing and triplet lambs were offered concentrate, to a maximum of 300g/day, from birth to weaning. Ewes rearing singles or twins received no concentrate post lambing. Lambs were weaned at 14 weeks of age. Strongyle faecal egg counts were determined for each ewe prior to the initiation of concentrate feeding at 6 weeks prior to lambing, lambing (within 3 days of lambing prior to turnout at pasture), and at 5, 10 and 14 weeks post lambing. Faecal egg counts were distinguished as *Nematodirus* (FEC_N), *Strongyloides papillosus* (FEC_S) and 'other trichostrongyles' (FEC_{OT}). Data were analysed using Proc Mixed with animal as a random effect. Prior to analysis, the FEC data were transformed to logarithms (ln (x + 1)) to stabilise the variance.

Results The effects of treatment on FEC_N, FEC_S and FEC_{OT} are presented in Table 1. There was no significant effect of treatment on FEC_N, FEC_S or FEC_{OT}. The effect of time on FEC_{OT} is presented in Figure 1 which shows that FEC_{OT} was elevated in late pregnancy and early lactation compared to other time points. There was a significant effect of time for all FEC variables (P<0.001).

Table 1 The effects of shearing treatment on faecal egg count – least square means (standard errors) on log scale

Treatment	FEC _N	FEC _S	FEC _{OT}
Conventional	0.1 (0.04)	1.0 (0.18)	4.3 (0.20)
Conventional and Pre-Mating	0.1 (0.04)	1.2 (0.15)	4.7 (0.20)
Pre_mating	0.1 (0.05)	1.0 (0.18)	4.6 (0.23)
Housing	0.1 (0.05)	1.1 (0.18)	4.6 (0.23)

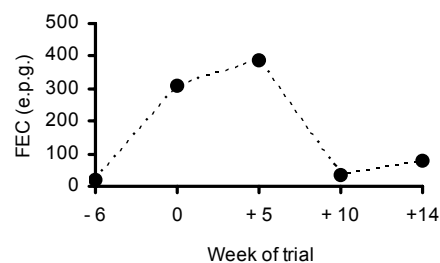


Figure 1 The effect of time on FEC_{OT} (back transformed least square means)

Conclusions The results indicate that the timing of shearing had no effect on the periparturient rise in nematode egg count in ewes. FEC_{OT} increased during late pregnancy and early lactation.

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Effects of periparturient metabolizable protein nutrition on ewes subjected to different infection pressures with the abomasal nematode *Teladorsagia circumcincta*

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Introduction Nematode egg excretion by periparturient ewes is the main source of infection for their immunologically naïve lambs. It has been shown that periparturient metabolizable protein (MP) supplementation can reduce nematode egg excretion (Houdijk *et al*, 2003). The latter experiment used a single moderate level of infection, but effects of MP supply on periparturient parasitism may depend on the level of infection. The objective of this study was to investigate the effects of MP supplementation on parasite control, ewe and lamb performance in ewes trickle infected with the abomasal nematode *Teladorsagia circumcincta* at three different infection levels. We hypothesised that the magnitude of beneficial effects of MP supplementation will be higher at the highest level of infection due to the expected nutrient drain on the host.

Materials and methods Seventy-two Greyface ewes, scanned for twin pregnancy at 9 weeks before mean achieved lambing (day₋₆₃), were infected with either 1000 (n=12), 5000 (n=12) or 10,000 (n=48) infective *T. circumcincta* larvae, every Mon-Wed-Fri from day₋₄₂ until day₂₅. From day₋₂₄, ewes of each group were restrictedly fed at 0.9 times their metabolizable energy requirement and either 0.8 (LP) or 1.3 (HP) times their assumed MP requirement (MPr), as per AFRC (1993) recommendation. Diets consisted of 1/3 chopped hay and 2/3 concentrates. Ewes and their lambs were weighed weekly and within 12h post lambing. Ewe faecal egg count (FEC, eggs per gram fresh faeces) was assessed twice weekly, and were transformed via log(FEC+1) for statistical analyses through repeated measures ANOVA. Post parturition ewe and litter body weight (BW), and litter body weight gain were analysed using ANOVA with ewe initial BW as a covariate.

Results Immediately post parturition, HP ewes were heavier than LP ewes (67.9 vs 65.4kg; s.e.d. 0.7kg; P=0.001) but this was not affected by level of infection or nutrition x infection interaction. These effects were maintained throughout lactation, with HP and LP ewes weighing 67.1 and 64.6 kg by day₂₅ (s.e.d. 0.9kg; P=0.007). Litter birth weight was not affected by the level of maternal MP nutrition, level of infection or their interaction and averaged (±s.d) 9.8±1.2kg. HP litters grew faster than LP litters (708 vs 651 g/d; s.e.d. 15g/d; P<0.001), but litter growth was not affected by level of infection or nutrition x infection interaction. Figure 1 shows the back-transformed mean FEC (with back transformed S.E.) during periparturient period. The effect of time on FEC tended to be significant both during pregnancy (P=0.091) and early lactation (P=0.055). The interaction effects between MP and level of infection were not significant for FEC during late pregnancy and early lactation. All first order and second order interactions with time were not significant during periparturient period. Late pregnancy FEC was affected by level of infection (P<0.001) but not by MP nutrition. In contrast, increased MP supply reduced FEC during lactation (P=0.015) but the level of infection only tended to be significant (P=0.097). Throughout the study, FEC decreased with increasing level of infection.

Conclusion Protein supplementation improved BW of both ewes and lambs. It reduced FEC during lactation which has an important implication for reducing pasture larvae contamination and thereby reducing infection risk for their naïve lambs during their early life. In contrast to our hypothesis, MP supply did not interact with level of infection, whilst the reverse relationship between FEC and the level of infection could be due to density-dependent effects elicited by the level of infection on parasite establishment and fecundity (Paterson and Viney, 2002).

Acknowledgements

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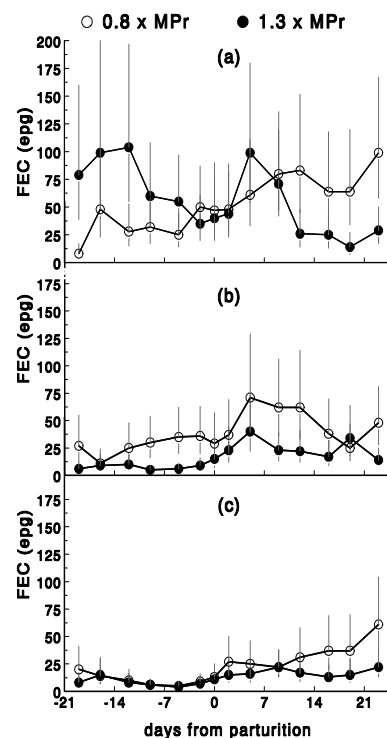


Figure 1 Back transformed faecal count (with 95% confidence interval) at (a) 1000, (b) 5000 and (c) 10000 L₃ of *T. circumcincta* per day for ewes fed at 0.8 (○) or 1.3 (●) times metabolizable protein requirement (MPr)

A study on the feasibility of using urea molasses block as a carrier of herbal anthelmintics against gastrointestinal nematodes in lactating cows

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Introduction In Bangladesh gastrointestinal nematode (GIN) infection is a chronic problem and is frequently associated with the low nutritional status of animals (Rahman and Mondal, 1983). Chemical anthelmintics are expensive and not readily available in rural areas. Herbal anthelmintics proved to be effective under this condition. In situations where treatment for parasite infection is necessary urea molasses block (UMB) can be modified to include appropriate dose of anthelmintics and therefore it has been suggested that medicated UMB can form an integral part of strategic parasite control programme as well as will upgrade the nutritional status of the animals (Knox and Wan Zahari, 1998).

Materials and methods Nine dairy cows, having 300 or more nematode egg counts per g of faeces, were selected for the study. They were allocated to three groups (A, B and C), each having three animals, based on milk production and parity. Three rations were formulated with rice straw, green grass, wheat bran and mustard oil cake as the basal ingredients and were supplied twice daily to three groups. Leaves of two herbal anthelmintics, Pineapple (*Ananas comosus*) and Neem (*Azadirachta indica*) were freeze-dried and ground and the required amount (200 mg/kg LW of cows) added to the ingredients of UMB and mixed. Group A (control) received UMB carrying no herbal anthelmintics. The UMB with neem leaves and those with pineapple leaves were fed to the animals of group B and C, respectively. Each block of UMB was weighing 2.5 kg and was fed to each cow for 5 days. Faeces from animals were collected on days 0, 7, 15, 30, 45 and 60 and examined for nematode egg counts using both Stoll's method as well as McMaster counting technique (Hansen and Perry, 1999). The data were analysed according to randomised block design (RBD) using MSTATC package.

Results The efficacy of different anthelmintic treatments in herbal fortified UMB in terms of the faecal egg count reduction percentage (FECR %) in cows is shown in Table 1. It can be seen from the table that throughout the experimental period the efficacy of both the herbal anthelmintics groups was significantly ($P < 0.01$) higher than that of the non-medicated UMB control group. Between the herbal anthelmintic groups pineapple leaves gave significantly ($P < 0.01$) higher efficacy than neem leaves at every faecal collection. There also appeared to be a progressive increase in the efficacy of treatment over time. Table 1 also shows that there were significant ($P < 0.05$) effects of herbal anthelmintics on milk yield and live weight gain of cows. The average daily milk yield was significantly ($P < 0.05$) higher in animals of both pineapple and neem groups than the control group. Between the herbal anthelmintics groups, the use of pineapple leaves resulted in significantly ($P < 0.05$) higher milk yield than neem leaves. There were no significant differences in milk protein content between the three treatments, however, pineapple leaves gave higher ($P < 0.05$) fat content of milk while control group having the lowest values for both fat and protein contents.

Table 1 Effect of herbal anthelmintics in split doses with urea-molasses block (UMB) on their efficacy and milk yield and composition of dairy cows on-station

Parameter	Dietary groups			SED	Significance
	Control	Neem	Pineapple		
Efficacies (%) against GIN on 60 th day	18.44 ^a	63.45 ^b	84.31 ^c	11.51	**
Average live weight change (g/d)	71.91 ^a	82.68 ^a	96.78 ^b	14.22	*
Initial average milk yield (L/d)	4.05	4.10	4.00	—	—
Average final milk yield (L/d)	4.75 ^a	5.10 ^b	5.45 ^c	0.28	*
Initial milk protein (g/100 g)	3.81	3.80	3.85	0.05	NS
Milk protein on day 60 (g/100 g)	3.93	3.89	3.98	0.08	NS
Initial milk fat (g/100 g)	4.30	4.31	4.30	0.12	NS
Milk fat on day 60 (g/100 g)	4.60 ^a	4.72 ^a	4.84 ^b	0.10	*

Conclusions Delivery of herbal anthelmintics through medicated UMB to lactating cows resulted in substantial reductions in faecal nematode egg counts and increased milk production of dairy cows. UMB with pineapple leaf had significantly higher efficacy, milk yield and milk fat contents than UMB with neem leaf. UMB may be a good carrier for herbal anthelmintics (pineapple or neem) and be fed to cattle for the treatment of nematode infection and increased productivity.

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An evaluation of quebracho (*Schinopsis quebracho-colorado*) tannin for the treatment of sheep gastrointestinal parasites

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Introduction Since their development, farmers have become heavily reliant on anthelmintics for the effective control of nematode parasites in their lambs and other grazing livestock. However, alternative approaches in the control of nematode parasites now need to be found due to the increasingly widespread development of anthelmintic resistance (Athanasidou *et al.*, (2001), Butter *et al.*, (2000)). This paper reports an *in vivo* study carried out to determine the effectiveness of quebracho tannins as an alternative to anthelmintics in the control of gastrointestinal nematode parasites in lambs.

Materials and methods Forty Blackface cross and Cheviot cross store lambs (mean live weight 34.3 ± 2.67 kg (S.D)) were brought inside one week before the experiment, ranked according to live weight, allocated to replicates of 8 animals according to ranking, and to one of 5 treatments within each replicate at random. Faecal samples were collected and faecal egg counts (FEC) made. The lambs were housed in groups of 8, bedded on straw, offered hay *ad libitum* and 1.2kg of sugar beet pulp per group twice daily with continuous access to clean fresh water. The experiment was carried out between 11 November 2006 and 10 December 2006. On day 1, lambs in groups 1 and 2 were dosed with 30g quebracho tannin, and lambs in group 3 with 60g quebracho tannin. Lambs in group 4 were dosed with 8ml of a conventional anthelmintic wormer, Valbazen (containing albendazole, Pfizer Ltd, Sandwich, Kent, UK). Lambs in group 5 were undosed Controls. On day 3 of the experiment lambs in group 2 were dosed again with 30g of quebracho tannin. Lambs were weighed weekly, FEC were made twice weekly on six lambs of each group. Faecal samples were stored at 4°C until they were examined in the laboratory, and the number of nematode eggs per gram of faeces determined using the Improved Modified McMaster Technique (Mairiead MacLennan, personal communication). The effect of treatment on FEC was analysed by one-way analysis of variance at each separate sampling time. Data were transformed ($\log(x+1)$) to stabilise the variance.

Results Back transformed means with their ranges are given in Table 1. The Control had the highest FEC. Lambs dosed with Valbazen had a lower FEC than the Control, with those dosed with quebracho tannins giving intermediate egg counts. Although not significant statistically, treatment with Valbazen wormer gave the best weight gain of 2.0 kg, (Table 2). There was the suggestion of a response to the tannin.

Table 1 Back transformed means and ranges of faecal egg counts per g fresh faeces. (n=6)

Treatment	Back transformed mean and range FEC/g fresh faeces at each sample time					
	Day 0*	Day 2	Day 4	Day 9	Day 16	Day 23
Control	558 (100-1350)	1092 (<50-4500)	750 (200-1900)	858 (300-2400)	742 (150-1250)	642 (250-1400)
30g tannin once	600 (<50-1250)	367 (<50-1300)	375 (100-1150)	533 (50-1350)	375 (250-850)	342 (50-750)
30g tannin twice	1017 (150-2200)	583 (<50-1950)	275 (<50-750)	325 (<50-1050)	575 (50-2200)	458 (100-1000)
60g tannin once	958 (350-1750)	242 (100-650)	267 (<50-650)	375 (150-800)	300 (50-850)	392 (50-700)
Valbazen wormer	1675 (200-4000)	283 (<50-700)	17 (<50-100)	17 (<50-50)	33 (<50-100)	25 (<50-100)

*Day 0 Before dosing

Table 2 Live weights and growth of lambs treated with quebracho tannins (QT) or a conventional (Valbazen) wormer (n=8)

	Control	30g QT once *	30g QT twice	60g QT once	Valbazen wormer	SEM
Initial weight (kg) (a)	31.4	31.1	31.6	32.7	31.4	1.58
Finishing weight (kg) (b)	32.3	31.8	32.8	34.2	33.4	1.22
Liveweight gain (kg) (b-a)	0.9	0.7	1.2	1.5	2.0	0.59

Conclusions It is concluded that the use of quebracho tannins could become an effective approach for the treatment of intestinal parasites, especially with the increasing prevalence of resistance to conventional anthelmintics. However, further work needs to be carried out on the dose level and dosing regimens with the use of quebracho tannins in commercial situations.

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The interactive effects of maternal nutrition and subsequent grazing on bioactive forage on lamb performance and parasitic status

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Introduction Metabolizable protein (MP) supplementation can reduce faecal egg count (FEC) in periparturient ewes, thus reducing pasture contamination for their lambs (Houdijk *et al.* 2006). In addition, lambs grazing on bioactive forage (chicory) had lower FEC than lambs grazing grass/clover (Athanasiadou *et al.* 2007). Both periparturient MP nutrition and grazing on chicory increase lamb performance but these approaches have been developed and investigated independently. The objective of this experiment was to assess their interactive effects on lamb parasitic status and performance. It was hypothesised that, although beneficial effects of maternal nutrition will be augmented with subsequent grazing on bioactive forage, the magnitude of the latter effect will be higher for lambs from unsupplemented ewes.

Materials and methods Forty-eight twin-bearing Greyface ewes, mated to Suffolk rams and scanned for twin pregnancy at 9 weeks before mean achieved lambing date, were trickle infected with *Teladorsagia circumcincta* at a dose of 10,000 infective larvae per day (three days per week) from 7 weeks before to 3 weeks after lambing. Thereafter, infection continued once weekly until week 10. Ewes were fed at 0.9 times their assumed metabolizable energy requirement, and either 0.8 (low protein (LP); n=24) or 1.3 (high protein (HP); n=24) times their assumed MP requirement (AFRC, 1993). Three weeks post-lambing, the ewes with their lambs were turned out onto a pasture which was low in parasite background ('safe pasture'). Ewes and lambs grazed this pasture for 12 weeks before moving into the experimental plots (referred to as day₀), where animals grazed either grass/clover or chicory plots, balanced for maternal LP and HP nutrition. Ewes were removed on day₁₀ whilst lambs continued grazing until day₆₂. Lamb body weight and faecal egg counts (FEC), expressed as eggs per gram faeces (epg), were assessed fortnightly from day₋₈₃ and day₋₅₀, respectively. Average daily weight gain (ADG, in g/d) on safe and experimental pastures was estimated by linear regression. FEC was transformed according to log(FEC+1) and analysed using a repeated measure ANOVA, whilst ADG was analysed using general ANOVA.

Results Figure 1 shows lamb body weight and back-transformed mean FEC (with 95% confidence interval). Pooled SE for body weight gradually increased from 0.21 on day₋₈₃ to 0.91 on day₆₂. Until day₀, HP lambs grew faster than LP lambs (174 vs 155 g/d; s.e.d. 9 g/d; P=0.028). At experimental pastures this effect on body weight remained but ADG was not affected by previous maternal protein nutrition (P=0.796). However, lambs grazing chicory grew faster than those grazing grass/clover (203 vs 161 g/d; s.e.d. 13 g/d; P<0.001). Maternal protein nutrition and forage type did not significantly interact for ADG (P=0.194). FEC of lambs was low on day₋₅₀, averaging 8 (7-9) epg (Figure 1). Thereafter, HP lambs had significantly lower FEC than LP lambs on day₋₃₆ (P=0.015). Time interacted with forage for FEC (P<0.001), as lambs on chicory had consistently, but not always significantly, lower FEC counts than those from grass/clover plots. In addition, FEC tended to increase faster after day₃₄ for the LP lambs grazing grass/clover than for the other three groups of lambs (P=0.086 for three-way interaction between time, maternal nutrition and forage type).

Conclusion The results of this study support the view that improved maternal protein nutrition and subsequent grazing of chicory have additive effects on parasite epidemiology, as indicated by differences in FEC, although the hypothesised interaction between maternal nutrition and chicory grazing on lamb performance were not observed. Lambs from protein supplemented ewes grew faster and had lower FEC, whilst subsequent grazing on chicory further improves production and reduces parasitism. Such combination of alternatives can be exploited in non-chemical worm control strategies.

Acknowledgements

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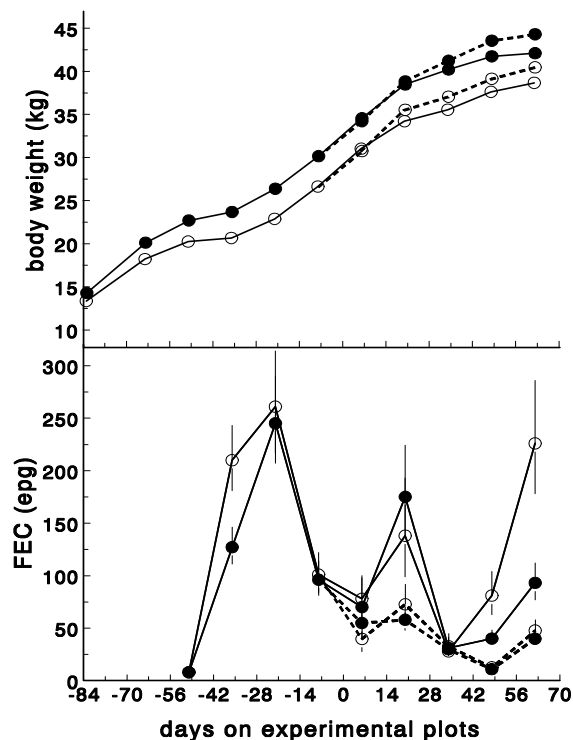


Figure 1 Body weight and FEC of lambs from protein supplemented (●) or unsupplemented ewes (○), and grazing grass/clover (solid line) or chicory (broken line) from day₀.

The effect of forage type, concentrate feed level and soyabean supplementation on the periparturient rise in nematode egg output in ewes

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Introduction A recent study at this Centre has shown that maize silage, regardless of maturity at harvest has the same feed value as grass silage when offered to ewes in late pregnancy (Keady and Hanrahan 2008). The control of gastrointestinal nematodes of sheep is largely dependent on the use of anthelmintics. The increasing evidence for resistance to nematode parasites of sheep to the broad-spectrum anthelmintics (Good, 2005; Good *et al.*, 2007) has led to the search for alternative sustainable solutions to parasite control. The temporary loss of acquired immunity to gastrointestinal nematodes that is indicated by an increased faecal egg output in late pregnancy and early lactation is a well established phenomenon and is important in the epidemiology of nematode parasites of sheep. There is substantial evidence for nutritional effects on the expression of immunity to gastrointestinal nematodes. In particular, there is evidence that faecal egg output of parasitised periparturient ewes is reduced in response to increased dietary metabolizable protein (Coop & Kyriazakis 1999; Donaldson *et al.*, 1998; Houdijk *et al.*, 2000). The objective of the present study was to evaluate the effect of diet during mid and late pregnancy as influenced by forage type and harvest system, concentrate feed level and soyabean supplementation on the peri-parturient rise in nematode egg output in ewes.

Materials and methods. Grass silage was harvested from the primary growth and regrowth of predominantly perennial ryegrass swards and ensiled either precision chopped or in big bales. Two maize silages were produced, either grown in the open [sown on May 10 (LDM)], or under the complete plastic mulch system [sown on April 10 (HDM)]. Each grass silage was offered *ad-libitum* supplemented with either 18 or 27 kg concentrate whilst the maize silages were offered *ad-libitum* and supplemented daily with either with 0 or 200g soyabean meal/ewe daily and 18 kg concentrate in late pregnancy (last 6 weeks). Ewes (Belclare x Scottish Blackface, Chamoise x Scottish Blackface) were housed (slatted pens) in mid December and had not been administered an anthelmintic in the previous year. The 12 treatments were offered to 180 ewes during mid and late pregnancy, penned in groups of five (three pens / treatment). Strongyle faecal egg counts (FEC) were determined from faecal samples collected from each ewe at 6 weeks prelambling, lambing (within 3 days of lambing, prior to turnout to pasture), and at 5, 10 and, 14 weeks post lambing. FEC were distinguished as *Nematodirus* (FEC_N), *Strongyloides papillosus* (FEC_S) and 'other trichostrongyles' (FEC_{OT}). Data were analysed using Proc MIXED of SAS with animal within treatment as a random effect. Prior to analysis, the FEC data were transformed to logarithms (ln (x +1)) to stabilise the variance.

Results As FEC_N and FEC_S were negligible, only the results of FEC_{OT} are reported. As linear contrasts, the effects of forage type, harvest system, harvest number, concentrate level and protein supplementation on FEC_{OT} are presented in Table 1. None of these factors were associated with a significant effect on FEC_{OT}. The effect of time on FEC_{OT} (back transformed least square means) is presented in Figure 1 which shows FEC_{OT} was elevated in late pregnancy and early lactation compared to the other time points.

Table 1 The effects of forage type, harvest system, harvest number, concentrate feed level and protein supplementation on FEC_{OT}

Treatment Contrasts	FEC _{OT}
Concentrate feed level 18 kg vs 27 kg	-0.02 (0.211) [†]
Harvest system : bale vs precision	-0.21 (0.211)
Maize type : low DM vs High DM	-0.49 (0.297)
Maize + Soya vs Maize, no Soya	-0.46 (0.297)
Forage type : Maize vs grass	-0.14 (0.264)
Harvest number 1 vs 2	0.03 (0.211)

[†] s.e. in parentheses

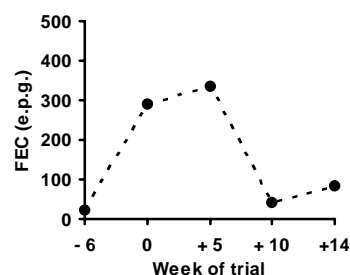


Figure 1 The effect of time on FEC_{OT}

Conclusions The results indicate that changing diet by concentrate level and protein supplementation had no effect on the peri-parturient rise in nematode egg count in ewes.

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Effects of intrinsic growth ability and protein nutrition on ability to cope with pathogens in mice

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Introduction Selection for narrow breeding goals, such as intrinsic growth ability, may reduce the animals' ability to cope with pathogen challenge (Rauw *et al.*, 1998). Using mice divergently selected for body weight, it has been shown that selection for high body weight reduced ability to cope with a parasitic challenge under low dietary crude protein (CP) conditions, whilst increasing protein nutrition from 50 to 250g/kg dietary CP reduced the penalty of infection in the high body weight line (Houdijk & Bünger, 2007). However, host resistance was not sensitive to protein nutrition, which may have been due to the observed increased food intake on the low CP diet and/or a reduction of food intake as a consequence of excess protein on the high CP diet. The current experiment addressed these assumptions through assessing interactive effects of intrinsic growth ability and infection at a range of levels of dietary CP contents.

Materials and methods Two levels of intrinsic growth ability and infection, and six levels of dietary crude protein content were used in a 2x2x6 factorial design (n=6). Male 'Roslin line' mice divergently selected for high (ROH) or low (ROL) body weight at 42 and 70 days of age over 28 generations (diverging in body weight by a factor of 2) were obtained (Bünger *et al.*, 2001). Mice were individually housed from weaning and given *ad libitum* access to 30, 80, 130, 180, 230 or 280 g/kg CP iso-energetic diets from 28 days of age. At 32 days of age, mice were either sham infected with water or infected with an intestinal nematode, *Heligmosomoides bakeri*, at a dose of 250 infective larvae. Feed intake and body weight were measured twice weekly until day 28 post infection, when colon egg counts were assessed as a measure of parasitism. Mean body weight gain, average daily feed intake, and log-transformed number of eggs in the colon were analysed using Restricted Maximum Likelihood (REML) analysis, to deal with a post hoc incomplete factorial design.

Results ROL mice fed 30g/kg dietary CP could not cope with infection past day 7, and had to be euthanised. Infection interacted with intrinsic growth ability for daily feed intake ($P<0.001$; Figure 1a). When infected, ROH mice showed a reduced feed intake when compared to their non-infected counterparts ($P<0.001$), this was not observed in ROL mice. At 30g/kg CP ROH mice ate less than on the other CP diets ($P<0.001$). The three way interaction between intrinsic growth ability, protein and infection was significant for daily weight gain ($P=0.017$; Figure 1b). Daily weight gain was reduced during infection in ROH mice ($P<0.001$) and this was affected by dietary protein content ($P<0.001$), however weight gain of surviving ROL mice was constant regardless of infection or dietary protein. ROH mice showed higher colon egg counts than ROL mice ($P<0.01$), but this did not interact with protein levels for either line (Figure 2).

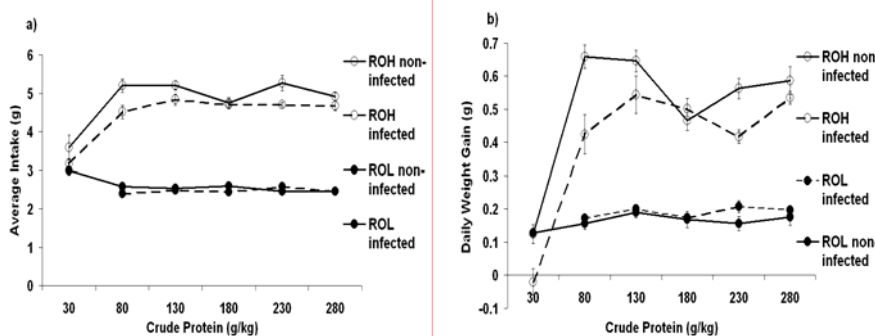


Figure 1 Mean daily food intake (a) and body weight gain (b) of high (ROH) and low (ROL) body weight mice during 28 days following infection with *H. bakeri* or sham infection with water at different levels of dietary CP.

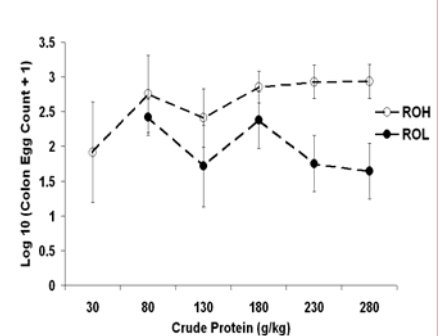


Figure 2 Mean number of eggs in colon of high (ROH) and low (ROL) body weight mice, at different levels of dietary CP.

Conclusion The results support the view that selection for intrinsic growth ability reduces the ability to cope with pathogen challenge, as the high body weight line (ROH) showed a greater reduction of body weight gain and feed intake during infection than the low body weight (ROL) line. ROH line mice also showed a higher level of parasitism, as indicated by colon egg count, coinciding with the increased penalty of infection. This penalty appears to be overcome at levels of dietary CP higher than 130 g/kg, whilst dietary CP contents did not appear to affect colon egg counts in either line. However, the inability of the ROL mice to cope with infection at low protein foods indicates that selection for intrinsic growth ability may change the way that animals cope with a pathogen challenge at times of nutrient scarcity.

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A study of feet lesions and locomotion scores in four crossbred ewe types

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Introduction Lameness is a major economic and welfare issue for the sheep industry in the UK and Ireland. It has been estimated that footrot costs the UK sheep industry £24 million per year (Nieuwhof and Bishop, 2005). Although a feet lesion scoring system in sheep has been devised to monitor lameness, it is a time-consuming process. Locomotion scoring is well established in dairy cattle to record the incidence of lameness, but needs validation within sheep. The primary aim of the present study was to investigate the association between locomotion score and the level and pattern of feet lesion scores in sheep. There is some evidence that there are differences in susceptibility to lameness between breeds. Therefore, a secondary aim of the study was to investigate feet lesions and locomotion in four crossbred genotype ewes.

Materials and methods The experiment was carried out over a period of three years on 4 lowland farms located across Northern Ireland. Four breeds of ewes were obtained from the study by Carson *et al.* (2001). These were Blue Leicester X Scottish Blackface (BLXB, n=310), Texel X Blackface (TXB, n=256), Suffolk X Cheviot (SXC, n=263) and Texel X Cheviot (TXC, n=246). Immediately before mating, all feet of each animal were scored for the presence and number of lesions in four areas of the foot as described by Morgan (1987). Additionally, a severity score of 0 to 4, ranging from 'no problems' (0) to 'excessive' (4), was given for each area. Additionally, ewes were assigned a score for locomotion score between 1 and 5, with '1' indicating an absence of lameness and '5' indicating extreme lameness (adapted from Fitzgerald *et al.*, 2000). Due to the unbalanced nature of the experimental design, the data were analysed using the GenStat REML (Residual Maximum Likelihood) procedure, with fitted fixed effects for year, farm and ewe genotype. Categorical data were analysed by binary or multinomial logistic regression using GenStat.

Results The association between feet lesion score and locomotion score was significant (odds ratio (OR) 1.07, 95% confidence interval 1.05-1.10, $P < 0.001$). Thus, as cumulative lesion score increased by a unit of 10, ewes were twice as likely to be lame. The number of lesions, severity of the lesion scores observed and total cumulative lesion scores for all feet were lower for the Suffolk X ewes compared with the other ewe breed types ($P < 0.05$) (Table 1). The number of lesions recorded was higher in the front compared with hind feet (OR 1.79, 95% confidence interval 1.59-2.01, $P < 0.001$). Significant breed differences were observed in the front feet ($P < 0.001$) (Table 1). Examining cumulative lesion scores in the different areas of the feet showed that only in the heel area were differences found in cumulative lesion scores between ewe genotype ($P < 0.05$). Overall, the incidence of lameness was lower for Suffolk X ewes compared to the Texel X ewes ($P < 0.01$).

Table 1 The effect of ewe genotype on hoof lesions and lameness

	BLXB	TXB	SXC	TXC	s.e.d.	P-value
<i>Number of lesions</i>						
All feet	2.52 ^b	2.34 ^b	1.88 ^a	2.21 ^{ab}	0.198	0.003
Front feet	1.67 ^c	1.53 ^{bc}	1.20 ^a	1.40 ^{ab}	0.114	<0.001
Hind feet	0.85	0.81	0.69	0.81	0.104	0.304
<i>Severity score[†]</i>						
	1.79 ^b	1.82 ^b	1.50 ^a	1.77 ^b	0.114	0.003
<i>Cumulative lesion score</i>						
Total feet	5.51 ^b	5.83 ^b	4.43 ^a	5.36 ^b	0.457	0.007
Interdigital (area 1)	0.04	0.06	0.11	0.07	0.041	0.511
Heel (area 2)	0.15 ^a	0.39 ^b	0.28 ^{ab}	0.41 ^b	0.120	0.033
Sole and abaxial wall (area 3)	1.05	1.44	1.24	0.95	0.190	0.077
Toe (area 4)	3.82	4.08	3.46	3.68	0.329	0.227
Lameness	0.13 ^{ab}	0.19 ^{bc}	0.10 ^a	0.22 ^c	0.031	0.004

Means within rows with different superscripts are significantly different ($P < 0.05$)

[†]The maximum severity score given for any lesions recorded on ewe's feet.

Conclusions A significant relationship between number and severity of feet lesions and locomotion score in sheep was found. This indicates that locomotion scoring could be a useful tool for lameness surveillance and monitoring within sheep flocks. These results also indicated that Suffolk X ewes had fewer lesions, lower severity of lesions, and less lameness overall compared to the other ewe genotypes.

Acknowledgements Financial support from DARDNI and AgriSearch is gratefully acknowledged.

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Prevalence of lameness in commercial dairy flocks of Chios sheep in Greece

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Introduction Sheep lameness is a major welfare concern as well as a challenge for sheep farmers because it is associated with reduced production and economic losses. The etiology of lameness can be either of environmental origin associated with housing, nutritional and genetic factors, or associated with the physiological changes during the productive life of sheep. Although, there is now a large body of research studies about lameness in meat producing sheep, such information in the literature regarding dairy sheep is very limited (Grogono-Thomas and Johnston, 1997; Winter, 2004). The Greek sheep industry is mainly based on milk production and is characterised by the recent establishment of many large, commercially run flocks. The aims of this study were to determine the prevalence of lameness (total number of cases at a given time) in such flocks of Chios sheep and to obtain data concerning the risk factors and type of management in relation to lameness.

Materials and methods The study involved 60 large commercial dairy flocks of the Chios Sheep Breeders Cooperative "Macedonia". The flocks had an average of 213 ewes and 296 litres of milk yield per ewe. The investigation was based on data obtained from a questionnaire, which was designed for in-depth interviews with the farmers and detailed recording of farm conditions. The information was collected from a single veterinarian following visits to individual farms between June 2007 and September 2007. The first section of the questionnaire had questions about the occurrence of lameness. Hence, all farmers were asked to recount the cases of lameness, indicate the months with the highest incidence and describe their treatment procedure over the previous year. The second section of the questionnaire comprised more general questions about management and feeding practices. Moreover, at the time of visit, the hygiene level of farm buildings was assessed to identify risk factors for lameness. Data sheets were devised to convert the descriptive information collected by questionnaires into a numerical form that could be used for statistical analyses. The association of lameness with various factors and farm practices was determined with a series of chi-square tests.

Results There was a significant prevalence ($P < 0.05$) of lameness in 22 of the 60 farms (36.7%). The perceived importance of lameness in animal performance, by farmers, was very high ($P < 0.01$). The majority of them (80%) considered lameness as the reason for reduced milk yield which was observed in the 71.7% of reported cases of lameness. Table 1 shows the main causes and the prevalence of lameness over one year. The highest prevalence of lameness was associated with foot rot followed by injuries and foreign bodies. In flocks where sheep were reared semi-intensively the prevalence of lameness was higher compared to those reared intensively ($P < 0.05$); it is most likely associated with the fact that foot trimming and foot bathing was performed routinely in some of the latter flocks. There was a significant correlation ($P < 0.01$) between type and adequacy of bedding material with both prevalence and intensity of lameness; farms using a combination of straw and sawdust for bedding had the lowest prevalence. However, stocking density did not have any significant effect on the prevalence of lameness.

Table 1 Etiology and prevalence of lameness in dairy flocks of Chios Sheep in Greece

Etiology of lameness	Number of farms	Prevalence
Footrot	35	58.3%
Injuries-foreign bodies	23	38.3%
Hoof overgrowth	9	15.0%
Scald	9	15.0%
Laminitis	2	3.3%
Trace element deficiency	1	1.7%
White line disease	1	1.7%

Conclusion Results showed that there is a considerable prevalence of lameness in dairy flocks of Chios sheep. The latter implies that control measures at farm level are either ineffective or not implemented correctly to be effective. Moreover, very few farmers undertake routine foot trimming or use footbathing and vaccinations. It is concluded that knowledge of the predisposing factors for lameness will assist farmers and veterinary surgeons in making a correct management plan. Further work is required to investigate and characterise the relationship between lameness and milk yield in dairy sheep flocks.

Acknowledgements The authors would like to thank the members of the Chios Sheep Breeders' Cooperative "Macedonia" for their help and contribution. The first author acknowledges financial support from the Greek State Scholarships Foundation.

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The efficacy of natural extracts of aloe vera (*Aloe barbadensis*), neem oil (*Azadirachta indica*) and tea-tree oil (*Melaleuca alternifolia*), as treatments for ovine footrot

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Introduction Ovine foot rot is a chronic, contagious, and painfully debilitating bacterial disease affecting the hooves of sheep. It is the most common cause of lameness, resulting in a loss of an estimated £24 million annually (Nieuwhof & Bishop, 2005). The main causative agents *Dichelobacter nodosus* and *Fusobacterium necrophorum*; two symbiotic, Gram-negative, strictly anaerobic bacteria. Treatment is usually by regular footbathing with formalin (3% v/v) or zinc sulphate (10% w/v), antibiotic injection, or by topical application of an antibiotic combined with paring of the hoof. Natural methods of treatment have the potential of circumventing the increasing problem of antibiotic resistance, in addition to being favoured by consumers due to their perceived health and environmental benefits. Some plant extracts are known to exhibit multicomponental antibacterial actions. This study investigated the efficacy and practicability as treatments for ovine foot rot of three plant extracts all of which have been shown in numerous studies, mostly carried out *in vitro*, to kill a range of bacteria, when applied topically.

Materials and methods Treatments were: 1) Gel Control. SPLF, a rheological sepiolite (Harbro Ltd, Turriff, Aberdeenshire), 7g SPLF in 100ml water, forming a brushable gel; 2) Aloe vera (*Aloe barbadensis*), 7g SPLF in 50ml water, plus 50ml aloe vera concentrate (whole leaf extract, Now foods, Bloomingdale, IL 60108, USA); 3) Neem (*Azadirachta indica*) seed oil, 7g SPLF in 100ml water, plus 10ml 100% pure Neem seed oil (Neemteam, Caerphilly, Wales); 4) Tea-tree (*Melaleuca alternifolia*) oil, 7g SPLF in 100ml water, plus 10ml 100% pure Tea-tree oil (Now foods, Bloomingdale); 5) Oxytetracycline antibiotic aerosol Terramycin™. Thirty-seven infected sheep were each allocated a treatment, at random, a replicate at a time. Treatment was carried out on a weekly basis on weeks 0, 1, 2, 4 and 5 over a five-week period. Treatments 1 – 4 were brushed on as the gel; treatment 5, was sprayed as the aerosol. On each occasion, each foot was scored (Table 1).

Table 1 Foot rot scoring system. Divisions of 0.5 were used and animal means of all four feet were analysed

Score	Description
	Modified from Egerton & Roberts, (1971), cited by Whittington & Nicholls, (1995).
0	Normal foot
1	Mild inflammation of the interdigital skin, moist, reddening and hair loss.
2	More extensive inflammation of the interdigital skin including tissue damage of the soft horn of the inner (axial) wall. Benign foot rot lesion.
3	Severe interdigital inflammation leading to under-running of the soft horn of the heel and sole. Virulent foot rot lesion.
4	Under-running, more severe tissue damage extending to the walls of the hoof.
5	Under-running of the hard horn of the outside (abaxial) walls of the hoof.

Results There was a clear response to each of the five treatments (Table 2), although only Tea-tree oil and Terramycin™ were significantly ($P < 0.05$) better than the Gel Control. The results are slightly confounded due to the low starting level of the Gel Control.

Table 2 Initial and final foot scores. The mean regression coefficients are of foot scores regressed against time

	Gel Control	Aloe vera	Neem	Tea-tree	Terramycin™	SEM
n = (sample size)	7	7	7	7	6	
Mean value week 0	1.50	3.29	2.43	4.86	2.92	0.690
Mean value week 5	0.13	0.93	0.79	1.94	0	-
Difference (weeks 0 and 5)	1.36	-2.36	-1.64	-3.00	-2.92	0.475
n =	8	7	7	8	7	
Regression coefficient	-0.30	-0.43	-0.37	-0.62	-0.65	0.090

Conclusions The results of this study, apparently the first to investigate the efficacy of plant extracts to treat ovine foot rot, provide encouragement for further research into the potential of these natural products for the treatment of foot rot which have particular relevance to organic production systems. It has not escaped our notice that the slight sealing effect of the Gel Control may have helped healing (assuming that the disease would otherwise have progressed normally).

Acknowledgements We are grateful to Harbro Ltd, Turriff, Aberdeenshire for the gift of the SPLF gel used in this work, and to Dr Graham Dalton for the use of his sheep.

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Estimation of genetic associations between piglet survival and performance traits for sire and dam lines with common ancestry

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Introduction Recently much research has been carried out to enhance piglet survival by genetic improvement. Heritabilities of traits associated with piglet survival are generally low, but the genetic variation is large enough to provide improvement through breeding (Knol *et al.*, 2002). However, correlations between some traits and/or between maternal and direct genetic effects have shown contradictory results, and not many studies have considered these effects. Reports of correlations between piglet survival traits and production traits are even fewer. The aim of this study was to estimate heritabilities of piglet survival traits and their genetic associations with other reproduction traits as well as production traits, using a Bayesian approach and appropriate models and genetic statistical procedures in order to obtain more accurate genetic parameters.

Materials and methods About 25 years ago the pig breeding company JSR separated their Large White breeding population into two different breeding lines: one line selected mainly for production traits (sire line) and a second line selected mainly for reproduction traits (dam line). Data sets consisted of information on both production traits (average daily gain (ADG) and back fat thickness (BF)) as well as reproduction traits (number born in total (NBT), number born alive (NBA) and percentage of piglets dead at birth (DB)) in both lines from 1991 until 2007. Fixed effects included batch, service type, parity, gestation length and weaning period. Crossfostering occurred in 42 % of the litters in the sire line and 37 % of the litters in the dam line. Crossfostering in the analysed population was registered as the number of piglets on- or off-fostered until weaning. The number of animals that died from birth till weaning did not distinguish between piglets from the biological mother and piglets fostered on from other dams. The data sets were analysed with REMLF90 (Misztal *et al.*, 2002) followed by a Bayesian analysis using GIBBS2F90 (Misztal *et al.*, 2002) and POSTGIBBSF90 (Misztal *et al.*, 2002).

Results Table 1 and Table 2 show the estimates of the heritabilities and genetic correlations for the mortality traits with performance traits. Heritabilities for reproduction traits were overall low. In general these heritabilities were slightly higher in the sire line than in the dam line except for DB. Genetic correlations of mortality traits with reproduction traits showed substantial variation, both within line and between lines. Genetic correlations were favourable for DB with NBT and NBA in the sire line and DB with NBA in the dam line. Heritabilities for production traits were moderate for ADG and high for BF. Correlations of DB with ADG were slightly favourable in the sire line but unfavourable in the dam line. In contrast, genetic correlations of DB with BF were undesirable in the sire line but slightly favourably correlated in the dam line.

Table 1 Heritabilities (diagonal), genetic correlations (above diagonal) and standard errors of correlations* (below diagonal) of the traits in the sire line

	DB	NBT	NBA	ADG	BF
DB	<u>0.03</u>	-0.04	-0.23	-0.01	-0.37
NBT	0.02	<u>0.16</u>	0.98	-0.01	0.07
NBA	0.02	0.00	<u>0.14</u>	-0.02	0.14
ADG	- na	- na	- na	<u>0.31</u>	0.29
BF	- na	- na	- na	0.00	<u>0.52</u>

* Standard errors of heritabilities were all < 0.002.

na Standard errors not available in the REML Program.

Table 2 Heritabilities (diagonal), genetic correlations (above diagonal) and standard errors of correlations* (below diagonal) of the traits in the dam line

	DB	NBT	NBA	ADG	BF
DB	<u>0.07</u>	0.24	-0.11	0.27	0.03
NBT	0.01	<u>0.12</u>	0.94	0.16	-0.09
NBA	0.01	0.00	<u>0.10</u>	0.06	-0.09
ADG	- na	- na	- na	<u>0.30</u>	0.07
BF	- na	- na	- na	0.00	<u>0.42</u>

Conclusions Heritabilities for survivability and reproduction traits were low, but genetic variation was substantial in these traits so that genetic improvement is expected to be efficient. Surprisingly, the heritability of BF was higher in the sire line than in the dam line although selection pressure has been mainly on this trait in the sire line. Selection pressure on litter size in the dam line may have resulted in the low unfavourable correlation between NBT and DB, which was slightly favourable in the sire line. Although the correlation between NBA and DB was favourable in both lines the dam line showed a lower estimate. Similarly, selection pressure on BF in the sire line may have resulted in the unfavourable correlation between DB and BF, which was slightly favourable in the dam line. This showed that selection has altered the correlations of the lines, which have developed from the same Large White breed. However, all unfavourable correlations between piglet mortality traits and reproduction traits or production traits were low so that simultaneous improvement of all traits can be achieved.

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Using novel partitioning methodologies to enable genetic parameter estimation for reproductive traits affected by porcine reproductive and respiratory syndrome virus (PRRSV) in pigs

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Introduction Predictive models have previously been applied to viral diseases such as porcine reproductive and respiratory syndrome virus (PRRSV) and have demonstrated that selection for resistance can reduce the likelihood of epidemics or the impact of disease on infected animals (Bishop and MacKenzie, 2003). Key components of selection programs for disease resistance are the characterisation of genetic variation and the identification of genetic markers or QTL associated with resistance to, or tolerance of, the pathogen. The growing evidence for genetic variation in host susceptibility to PRRSV has been described in the review by Lewis *et al.* (2007). This study seeks to develop data partitioning methodologies for describing the impacts of disease on pig performance, and estimate heritabilities traits affected by PRRSV.

Materials and methods The dataset analysed was from a pig multiplication herd from a commercial breeding stock company. The data consisted of reproductive performance trait records collected on all sows over a ten year period in which two PRRSV outbreaks were reported. We used two methods of separating the data into disease D and baseline B phases; these consisted of using a date threshold (DT), guided by veterinary diagnoses and a data threshold (TT), guided by observed changes in pig performance. The date threshold was defined from the 1st day of the month of outbreak (from random ELISA testing on farm) and then moving back 5 months to account for a full gestation period, a standard lactation period, sow dry time and the ca. 10 days that a sow usually takes to seroconvert post infection. The TT method consisted of selecting a relevant trait of interest (i.e. mummified piglets in the case of PRRS) during a normal (non-diseased) time period on the farm, calculating μ and σ , and defining the 99% confidence interval (CI) for this trait. The 30 day rolling average for mummified pigs was calculated and date thresholds were defined when the rolling average crossed the upper 99% CI value. From these thresholds we created a healthy baseline dataset (BP) and two disease-phase datasets DT and TT. Dataset BP consisted of 1019 litters from 110 sows, DT consisted of 1622 litters from 169 sows, and TT consisted of 1526 litters from 167 sows. There were 821 sows in common between BP and the two diseased datasets. Data analysed included number of matings required per conception, total piglets mummified, born dead and born alive, by sex. Total services, born dead and mummified were also analysed as binary traits, with appropriate thresholds. Fixed effects of sow line and sow parity were generally significant and fitted in all analyses. Genetic parameters were estimated using ASREML fitting an animal model (sire model for binary data) including all known pedigree relationships, which itself comprised 4104 animals. Random effects fitted included the direct genetic and the permanent environmental effect of the dam.

Results The results of the genetic parameter estimation are shown in the table below (top: raw data, bottom: binary data).

Trait	B phase			DT phase			TT phase			Mean values for traits associated with the PRRS outbreak, e.g. mummified piglets, increased during the disease phases as did the phenotypic SD (data not shown). Heritabilities also increased during the disease phase in the production traits, hence genetic
	Mean	h^2	SE	Mean	h^2	SE	Mean	h^2	SE	
Mating / Service	1.91	0.06	.01	1.27	0.40	.02	1.25	0.66	.02	
Mating / Concept.	2.25	0.04	.01	1.50	0.18	.02	1.48	0.46	.04	
Total <i>in-utero</i>	11.1	0.20	.01	11.1	0.16	.02	11.0	0.22	.04	
Total born alive	10.2	0.17	.02	9.26	0.05	.02	8.74	0.15	.03	
Total born dead	0.82	0.05	.01	1.82	0.01	.01	2.27	0.10	.03	
Total mummified	0.22	0.03	.01	0.94	0.01	.01	1.34	0.10	.03	
Total stillborn	0.60	0.04	.01	0.88	0.03	.01	0.93	0.08	.03	

Trait	Threshold level	B phase		DT phase		TT phase		SE
		h^2	SE	h^2	SE	h^2	SE	
Services	2	0.67	.29	0.47	.31	0.48	.18	
Total B.D.	1	0.32	.12	0.15	.12	0.32	.11	
Total stillborn	1	0.46	.16	0.16	.12	0.18	.09	
Total mummified	1	0.21	.12	0.28	.16	0.55	.14	

variances increased more than environmental variances.

Conclusions It is clear from the results that PRRSV has a major impact on reproductive trait means and heritabilities. Further, the way the data is partitioned into disease and baseline phases can markedly affect genetic parameter estimates. The methods used here could be a model for further population-level host/pathogen interaction studies in the future. In terms of PRRSV resistance, these results are encouraging as increased reproductive trait heritabilities were generally moderate to high, especially in the more stringent TT dataset, and often higher than during the healthy phase. If underlying markers/QTL could be identified that explain this phenotypic variation (e.g. via a SNP association study that we are currently undertaking) then breeding animals for reduced PRRS impacts could be possible in the near future.

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Genetic analysis of a two generation selection experiment under outdoor conditions designed to disentangle direct and maternal genetic effects of piglet survival

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Introduction Piglet survival is based on a complex interaction between the piglets own genetic component (direct genetic effects), the dams genetic contribution (maternal genetic effects) and environmental effects (systematic environmental such as year-season, common litter and individual environmental effects). Disentanglement of direct and maternal genetic effects needs a powerful design of genetic relationships. In order to accomplish this, a two generation selection experiment was designed with different selection groups for direct and maternal effects and cross-classification of these selection groups. Survival at birth and survival during the nursing period may have genetically independent components and would then be treated as different traits. In addition, piglet survival traits are reported to have low direct and maternal heritabilities and traits genetically associated with survival, such as birth weight, may result in a more efficient change in survival than using survival per se. Therefore, the objective of the research was to estimate the genetic parameters of direct and maternal genetic effects of survival and birth weight in order to enhance the selection strategies for piglet survival.

Materials and methods Data from 22,483 piglets born under outdoor conditions and recorded individually for survival and birth weight were used in the genetic analysis. Data were from a two generation selection experiment in which the first generation sires (Landrace) were selected in groups of high and average estimated breeding values (EBVs) of maternal genetic effects of piglets survival to produce gilts for the second generation. In the second generation, sires (Large White) were selected for high and average EBVs of direct genetic effects of piglet survival to obtain groups of piglets influenced differently by this genetic component. In the first parity of the second generation matched matings within corresponding high or average EBV groups for direct and maternal were carried out, whereas in the second and third parity cross-classified matings between all selection (EBV) groups were designed. This design was chosen to improve the power to disentangle direct and maternal genetic effects of piglet survival. Multiple trait Bayesian analysis using THRGIBBSF90 (Misztal *et al.*, 2002) was carried out in which a threshold model was fitted for survival traits and a linear Gaussian model for birth weight.

Results The estimated direct heritabilities were of moderate magnitude and substantially higher than reported in the literature (Table 1). Maternal genetic heritabilities of these traits were substantially lower and in particular for birth weight similar to those reported in the literature. Direct as well as maternal genetic correlations between survival traits were consistently low indicating their different genetic background. Direct or maternal genetic correlations between survival traits and birth weight were also lowly related, however, their associations were consistently favourable. In contrast, the genetic correlations between direct and maternal genetic effects within each trait were moderate and unfavourable in the range from -0.34 to -0.47 for the analysed traits. Additionally, direct and maternal genetic correlations among traits were unfavourable but of low magnitude.

Table 1 Direct and maternal heritabilities (h^2 , diagonal) and genetic correlations (above diagonal) and 95% highest posterior density interval (HPD, below diagonal or for h^2 last row) for survival at birth (SVB) and survival during nursing period (SVNP) and individual birth weight (IBW) using Bayesian analysis

Effect	Trait	Direct genetic effects (DIR)			Maternal genetic effects (MAT)		
		SVB	SVNP	IBW	SVB	SVNP	IBW
DIR	SVB	0.21	0.08	0.17	-0.36	-0.04	-0.09
	SVNP	-0.18 to 0.35	0.24	0.16	-0.01	-0.45	-0.06
	IBW	0.02 to 0.32	0.01 to 0.31	0.36	-0.05	-0.10	-0.36
MAT	SVB	-0.58 to -0.15	-0.26 to 0.25	-0.22 to 0.10	0.15	0.09	0.06
	SVNP	-0.26 to 0.21	-0.62 to -0.28	-0.25 to 0.05	-0.13 to 0.30	0.14	0.17
	IBW	-0.23 to 0.06	-0.20 to 0.08	-0.45 to -0.27	-0.08 to 0.20	0.04 to 0.30	0.28
HPD h^2		0.14 to 0.28	0.14 to 0.35	0.31 to 0.41	0.10 to 0.19	0.10 to 0.18	0.24 to 0.31

Conclusions The reasons for the high direct heritabilities may be due to multiple factors such as husbandry under outdoor conditions, crossing of different lines, creation of different selection groups and use of a threshold model in comparison to linear models fitted in other studies, etc. The low genetic correlations between survival at birth and during the nursing period indicate the need for the use of a multiple trait model for these traits. Moderate antagonistic relationships between direct and maternal genetic effects within each analysed trait are expected to have an important influence on overall genetic improvement of piglet survival. Low but favourable genetic correlations between birth weight and survival traits suggest that birth weight can be used to improve survival of piglets over and above selection for piglet survival per se.

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Genetic aspects of resistance to footrot in sheep

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Introduction Footrot is an endemic disease of sheep, costing the British sheep industry an estimated £24M annually (Nieuwhof and Bishop, 2005). Estimates for the heritability of footrot in Australian data are as high as 0.3 (Raadsma *et al.*, 1994), but this may differ in Britain because of different bacterial strains, sheep breeds and environmental conditions. The aim of this study is to determine the heritability and repeatability of resistance to footrot in Scottish Blackface (SBF) and mule sheep at different ages, and to assess if breeding for resistance to footrot is a credible option for British sheep breeders.

Materials and methods Using the method of Conington *et al.* (2008), in which each hoof is given a score ranging from 0 (healthy) to 4 (severe footrot), 4,340 SBF ewes from experimental and commercial flocks were scored for footrot once a year in 2005 and/or 2006, 726 mules from experimental flocks were scored on up to four occasions over 2 years and 1,199 SBF lambs were scored in 2005 only. Derived traits, and their names, were as follows. *FR* and *FR24* were defined as binary traits, with value 1 if at least 1 hoof had a score >0 or >1, respectively, and 0 otherwise. *FRsum* was the sum and *FRmax* the maximum of scores over 4 hoofs. Repeated measures were considered as expressions of the same trait, and analysed with ASReml (Gilmour *et al.*, 2002) using a model including fixed effects for management group, scorer, litter size, age, scoring occasion and significant interactions, as well as direct genetic and animal permanent environmental effects. The model for lambs included weaning weight rather than litter size and no permanent environmental effect. *FR* and *FR24* were analysed using a sire model with a logit link function ('threshold' model) and a linear animal model. Alternatively, repeated scores were considered as being different traits and the correlations were calculated using multivariate animal model analyses.

Results Average prevalence of footrot in SBF ewes based on *FR* was 0.17 in 2005 and 0.18 in 2006, in SBF lambs it was 0.34 and in mules it ranged from 0.27 to 0.64 (mean = 0.51) over the 4 scores. Table 1 shows estimates of the heritability of various footrot traits in ewes, which ranged from 0.06 to 0.26. Permanent environmental effects were always smaller, so that repeatabilities were low. Heritability estimates tended to be higher within year for SBF and within scoring occasion for mules (comparing Table 1 to Table 2). They were also higher when threshold models were used. In SBF ewes, genetic correlations for the same trait over two years ranged from 0.18 to 0.39 and phenotypic correlations from -0.17 to 0.07. In mules, genetic correlations between various foot

Table 1 Estimates of permanent animal effect and heritability (s.e.) in SBF ewes and mules, depending on model used

Trait	Model	SBF		Mules	
		c_{animal}^2	h^2	c_{animal}^2	h^2
FR	Threshold	0.04	0.19 (0.07)	0.10	0.12 (0.06)
	Animal	0.00	0.08 (0.02)	0.02	0.11 (0.06)
FR24	Threshold	0.07	0.26 (0.11)	0.14	0.19 (0.10)
	Animal	0.01	0.05 (0.02)	0.01	0.13 (0.07)
FRsum	Animal	0.03	0.06 (0.02)	0.09	0.11 (0.06)
FRmax	Animal	0.02	0.06 (0.02)	0.10	0.12 (0.06)

assessments (trait was *FR*) ranged from -0.10 to 0.87, albeit with large standard errors, and phenotypic correlations ranged from 0.06 to 0.22. All footrot traits in lambs had estimated heritabilities of zero.

Table 2 Estimates of heritability (s.e.) for SBF ewes and mules within scoring occasion (2 scores per year for mules), *FR* and *FR24* based on a threshold model, *FRsum* and *FRmax* based on an animal model

Trait	SBF 2005	SBF 2006	Mules		Mules	
			2005(1)	2005(2)	2006(1)	2006(2)
FR	0.26 (0.14)	0.21 (0.10)	0.10 (0.09)	0.26 (0.15)	0.20 (0.18)	0.13 (0.20)
FR24	0.61 (0.23)	0.25 (0.14)	0.09 (0.14)	0 (0)	0 (0)	0.59 (0.39)
FRsum	0.19 (0.06)	0.04 (0.03)	0.08 (0.08)	0.16 (0.11)	0 (0)	0 (0)
FRmax	0.16 (0.06)	0.05 (0.03)	0.07 (0.08)	0.17 (0.11)	0.09 (0.11)	0.08 (0.17)

Conclusions Resistance to footrot has a low to moderate heritability in SBF ewes and mules, while correlations between successive scores and repeatability are generally positive but low. Selection for resistance to footrot is therefore possible, but would greatly benefit from repeated measures on individuals and relatives. Resistance to footrot was found not to be heritable in lambs.

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Analysis of genetic relationships among footrot and performance data in Scottish Blackface sheep

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Introduction The presence of footrot in a flock can have serious implications for animal health, welfare and productivity. Associated increases in treatment and prevention costs, as well as losses in performance, have led to a notable interest in using breeding to control footrot in sheep. Current breeding indexes for UK hill sheep such as the Scottish Blackface, include both lamb and maternal traits, and have led to improvements in economic returns. So that breeding for resistance to footrot can be evaluated in the context of multi-trait selection programmes, the genetic relationships with current breeding goal traits must be quantified. The objective of this study was to identify important environmental factors affecting footrot prevalence and to estimate genetic parameters between footrot severity and other traits used in current UK hill sheep breeding indexes.

Materials and methods On two SAC farms, a total of 2,091 foot score records were collected in the autumns of 2005 and 2006 from 1383 Blackface ewes (708 of which were scored in both years). Each hoof was awarded a foot score ranging from 0 (healthy foot) to 4 (severe footrot) according to the method of Conington *et al.* (2008). The scores given to each of the 4 hooves of the animal were then summed and log-transformed (*LnFSum*). These data were matched up with performance data from a total of 18,151 lambs and 13,900 ewes, as well as pedigree data from a total of 21,299 animals. Ewe traits included: pre-mating live weight (kg; PMWT), age of the ewe at cull or death (years; LONGV), number of lambs the ewe has lost between birth and weaning (LLOST), number of her own lambs reared to weaning (LWEAN), maternal ability measured by total weight of lambs reared to weaning, divided by litter size (kg; MATWWT) and fleece weight (kg; FLWT). Lamb traits included: weaning weight (kg; WWT), ultrasound fat (UFD) and muscle (UMD) depths, carcass weight (kg; DCWT), Meat and Livestock Commission carcass fat grade (MLCF) and conformation grade (MLCC) at slaughter. The data were analysed using ASREML (Gilmour *et al.* 2002). Univariate heritability analyses included the fixed effects of farm, grazing area, ewe age, score year and litter size reared to weaning, and the direct animal genetic effect. Bivariate analyses among *LnFSum* and the performance traits were undertaken using the models described in detail by Lambe *et al.* (2007).

Results Predicted means for the fixed effects mentioned above found that the prevalence of footrot in the population differed significantly ($P < 0.05$) between years (2005 = 0.29, 2006 = 0.13) and between farms (Farm 1 = 0.13, Farm 2 = 0.29). Univariate heritability for *LnFSum* was low at 0.04, with a phenotypic variance of 0.21. The genetic and phenotypic correlations estimated between *LnFSum* and the ewe and lamb traits are shown in Table 1.

Table 1 Genetic (r_g) and phenotypic (r_p) correlations between foot score trait *LnFSum* and current index or goal traits

Ewe Trait	LnFSUM				Lamb Trait	LnFSUM			
	r_g	s.e.(r_g)	r_p	s.e.(r_p)		r_g	s.e.(r_g)	r_p	s.e.(r_g)
PMWT	0.16	0.16	0.002	0.02	WWT	-0.06	0.19	-0.01	0.02
LONGV	-0.11	0.62	0.00	0.02	UFD	-0.23	0.18	-0.02	0.02
LLOST	0.12	0.31	0.02	0.02	UMD	-0.33*	0.17	-0.04*	0.02
LWEAN	-0.57	0.32	0.04	0.03	DCWT	-0.15	0.25	-0.01	0.02
MATWWT	0.02	0.18	-0.04	0.02	MLCF	-0.20	0.26	-0.02	0.02
FLWT	0.34 *	0.16	0.051 *	0.02	MLCC	0.20	0.27	0.02	0.02

(* indicates correlations that are significantly different from zero; $P < 0.05$)

Conclusions Key factors affecting footrot prevalence may include breed-type, weather conditions and grazing management. The footrot heritability was low in this dataset. The genetic correlation estimates among footrot prevalence and performance traits were generally low and non-significant, apart from those with ewe fleece weight and lamb ultrasonic muscle depth. Moderate, but non-significant, correlations were estimated with number of lambs weaned by the ewe, lamb ultrasound fat depth and MLC fat and conformation grades. The inclusion of footrot into multi-trait breeding programmes is unlikely to affect the performance in other traits significantly. Further analyses estimating correlations among footrot and performance traits is currently underway using flocks with higher footrot prevalence, where the heritability may be higher (Nieuwhof *et al.* 2008), and different breeds.

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Genetic evaluation of female fertility in Ayrshire, Guernsey, and Jersey dairy cattle

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Introduction Fertility has been shown to have an unfavourable genetic relationship with milk production in Holsteins so continued selection for milk production will exacerbate the decline in fertility in the absence of information on daughter fertility. The only way to reverse this genetic trend is to breed dairy cows additionally for fertility. But dairy farmers have historically had no access to fertility information when they purchase semen. It is therefore vital that fertility is included in selection programmes and that farmers can choose to inseminate their cows with semen from bulls with a positive predicted transmitting ability (PTA) for daughter fertility. Although of low heritability, fertility traits have been shown to have sufficient genetic variation and have recently been made available nationally for the Holstein-Friesian breed (Wall *et al.*, 2003). The aim of this study was to investigate the feasibility of estimating fertility indices for Ayrshire (AYR), Guernsey (GUE) and Jersey (JER) dairy cows.

Material and methods Fertility and production information were extracted from the National milk recording organisations' databases. In summary the data available included direct measures of fertility including calving interval (CI), days to first service (DFS), non-return (conceived) 56 days post first insemination (NR56) and number of recorded inseminations for cows with a second calving (INS), and an indirect fertility measure of milk yield at day 110 of lactation (MILK). The data were validated and edited according to the rules applied for the Holstein breed (Wall *et al.*, 2003). Insemination, calving and milk yield information was extracted for first lactation animals including data until the end of May 2007 for GUE, AYR and JER cattle along with a three generation animal pedigree. Multi-trait BLUP breeding values were estimated with a linear animal model using the same methods and genetic parameters currently applied to routine national evaluations of fertility in Holstein-Friesian cattle (for further details see Wall *et al.*, 2003).

Results Table 1 describes the phenotypic data for each of the 3 breeds. The JER had a lower mean CI than the other breeds (389 days vs. 395 days) with a significantly lower CI than the AYR and GUE breed. The JER were also significantly younger at first calving than the AYR and GUE breed. There were significant differences between all three breeds in terms of the number of inseminations per successful calving (INS) with the JER breed requiring more services per calving, followed by the GUE and AYR requiring the least services per calving. The mean, standard deviation and range of sire PTAs for the fertility traits (and fertility index), after base adjustment, are presented in Table 2 for the 1,065 AYR bulls, 784 GUE bulls and 1,604 JER bulls and with daughters in the analysis dataset and a fertility index reliability of 10% or more. These results are presented on their own specific breed base and are therefore not comparable across breeds. Table 2 shows that bull PTAs for CI had a range of 19 days for AYR, 34 days for GUE and 24 days for JER but 95% of the observations lie in the range of -4 to +4, -5 to +5 and -5 to +5 respectively. NR56 had a range of 36.2 for AYR, 14.5 for GUE and 28.6 for JER meaning the difference between the worst and the best bulls for NR56 is that 36% more daughters in AYR, 14.5% in GUE and 29% more daughters in JER do not return to service within 56 days.

Table 1 Summary statistics of GUE, JER and AYR data (May 2007) used in BLUP runs.

	AYR		GUE		JER	
	Rec %	Mean (sd)	Rec %	Mean (sd)	Rec %	Mean (sd)
CI (days)	74	394.91 (52.42)	74	395.51 (53.68)	75	389.15 (53.44)
MILK (kg)	100	19.07 (4.65)	100	16.75 (4.57)	100	16.16 (4.44)
DFS (days)	89	85.04 (31.65)	92	79.86 (29.51)	93	82.12 (29.87)
NR56 (0/1)	89	1.70 (0.46)	92	1.64 (0.48)	93	1.68 (0.47)
INS (count)	70	1.61 (0.94)	71	1.77 (1.14)	73	1.65 (1.00)

Table 2 Range of fertility proof PTAs and overall fertility index in AYR, GUE and JER breeds

		CI	DFS	NR56	CINS	FI
		Mean	sd	Mean	sd	Mean
AYR	Mean	-0.48	-0.49	0.25	-0.00	0.55
	sd	2.39	1.78	2.05	0.04	3.54
	Range	19.20	36.72	36.24	0.63	55.55
GUE	Mean	-0.34	-0.19	0.72	-0.01	1.22
	sd	2.98	1.41	1.75	0.06	3.346
	Range	34.12	11.52	14.48	0.71	30.37
JER	Mean	-0.65	-0.48	0.26	-0.00	0.61
	sd	2.63	1.56	2.05	0.04	3.64
	Range	23.56	12.99	28.63	0.43	45.97

Conclusions This study shows that routine extraction and genetic evaluation of female fertility in non-Holstein dairy cows is possible. Initial estimation of breeding values show that the female fertility traits in the three breeds show genetic variation and therefore will allow the breeds to include fertility in their future selection decisions.

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Heritability of digital dermatitis and its association with locomotion, production, fertility and lifespan

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Introduction Digital dermatitis (DD), an infectious skin disease of cattle, is considered an important cause of lameness in dairy herds due to the resulting pain, discomfort and impaired performance (Read and Walker, 1998). DD has been associated with reduced fertility and a shorter lifespan (LS) as well as a decrease in milk production and generally reduces the overall wellbeing of the cows. As part of the UK type classification scheme, field officers record whether or not cows exhibit signs of DD during routine type evaluation. The aim of this study was to estimate the heritability of DD and genetic associations between DD, type traits related to feet and legs, with fitness-related traits and production.

Materials and methods Data comprised 93,391 national type evaluation records of first lactation Holstein-Friesian cows from 4,571 herds calving from 2002 to 2006. DD was scored as the presence (1) or absence (0) of lesions in the interdigital spaces of the feet. Three type traits; locomotion score (LOCO), bone quality (BONEQ) measured on a scale of 1 to 9, and leg and feet composite (L&F) measured on a subjective scale of “poor” (65) to “excellent” (95), were also evaluated. To obtain genetic parameters for DD and genetic correlations between DD and the other type traits, a series of bivariate animal model analyses, using ASREML (Gilmour, *et al.*, 2000), were performed. The heritability of DD was first estimated with this linear animal model and then transformed to an assumed underlying normally distributed scale (Robertson and Lerner, 1949). Approximate genetic correlations among DD, type traits, LS, production (milk and fat) and fertility traits (calving interval (CI) and non-return after 56 days (NR56), scored as 0 if the cow returns to service after 56 days and 1 otherwise) were estimated by correlating sire breeding values (EBV). Sire EBVs for DD and the other traits were taken from the output of the genetic analysis and reliabilities estimated from the standard errors of sire solutions. Sire EBVs for milk, fat, CI, NR56 and LS were taken from the UK national evaluation records. The low heritability of DD resulted in many bulls having predicted breeding values of low reliability. The minimum reliability for DD was therefore set at 0.10, which gave 2,461 observations and a lower reliability limit of 0.28 (LS), 0.41 (production traits) and 0.27 (fertility traits). The minimum reliability used for the type traits was 0.30 resulting in a minimum reliability of 0.50 (production traits), 0.34 (CI), 0.35 (NR56) and 0.32 (LS). Correlations among type and the other traits were based on 973 observations. Correlations of EBVs were adjusted by their reliabilities to obtain approximate genetic correlations by the method of Calo *et al.* (1973). The standard errors of correlations were estimated using the formula described by Sokal and Rohlf (1995).

Results The genetic variance of DD was significantly greater than zero. The overall incidence of DD was 12% resulting in the heritability of DD on the normal scale (0.029) being twice that on the linear scale (0.011) (Table 1). BONEQ, LOCO and L&F had moderate to high genetic correlations with DD (-0.21, -0.67 and -0.63, respectively). Table 1 gives the approximate genetic correlations between LOCO, BONEQ, L&F, DD and LS, production and fertility. The type traits were found to be strongly correlated with LS indicating that cows with a higher locomotion score, finer bones and better legs and feet last longer in the herd. Correlations between type, production and fertility were low. DD was moderately associated with LS (-0.16), milk (-0.31) and fat (-0.43). The correlations between DD, CI (-0.07) and NR56 (0.48) showed that an increased incidence of DD was associated with a shorter CI and a higher conception rate.

Table 1 Heritability (h^2) of DD and correlations with lifespan, production and fertility with their standard errors (SE)

Trait	h^2	SE (h^2)	LS	Milk	Fat	CI	NR56
DD	0.011 (linear)	0.003					
	0.029 (normal)	0.007					
LOCO	-		0.66 (0.02)	-0.04 (0.03)	0.22 (0.03)	0.04 (0.03)	0.02 (0.03)
L&F	-		0.69 (0.02)	-0.11 (0.03)	0.12 (0.03)	0.06 (0.03)	0.07 (0.03)
BONEQ	-		0.50 (0.03)	-0.12 (0.03)	-0.01 (0.03)	0.07 (0.03)	0.36 (0.03)
DD	-		-0.16 (0.02)	-0.31 (0.02)	-0.43 (0.02)	-0.07 (0.02)	0.48 (0.02)

Conclusions DD is heritable and the results show that cows with higher locomotion score, flatter, more refined bones and better leg and feet composite were less prone to DD. A higher locomotion score, flatter, more refined bones and better legs and feet are associated with a longer lifespan. A higher incidence of DD is associated with reduced longevity and a decrease in production.

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Toward a new practical UK energy evaluation system for dairy cows

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Introduction Energy evaluation systems translate animals' requirements for net energy (NE) into feed metabolisable energy requirements (MER). The Feed into Milk (FiM) project resulted in a new system for predicting the MER of dairy cows (Thomas, 2004). In contrast to AFRC (1993), the FiM system is (almost) independent of feed quality, like the system proposed in 1994 by Tolkamp & Ketelaars (T&K). To translate NE requirements into MER, FiM requires nine parameters, while for all cattle that are fed (near) *ad libitum* T&K require only one. Here we analyse how these parameters contribute to the differences in prediction of daily MER between the two systems and what the causes of these differences are.

Materials and methods In FiM, basic NE and ME requirements (MJ/d) for maintenance are $NE_m = 0.453 \times W^{3/4}$ and $ME_m = 0.647 \times W^{3/4}$. NE requirements for milk yield (MY, kg/d) are $NE_l = 3 \times MY$ MJ/d, corrected for cows with negative W change (ΔW , kg/d) to $NE_l = 3 \times MY + 0.78 \times 19.3 \times \Delta W$ MJ/d. MER (MJ/W^{3/4}) are calculated after dividing NE_l with $W^{3/4}$ as: $MER = (\log_e((5.06 - NE_l)/(5.06 + 0.453)))/-0.1326$. If $k_1 = NE_l/(MER - 0.647) < 0.59$, MER is corrected to $MER = NE_l/0.59 + 0.647$. After multiplication with $W^{3/4}$, an activity allowance is added of $0.0013 \times W/k_m$ MJ/d, with $k_m = 0.019 \times M/C + 0.503$ and M/C equal to the ME yield of 1 kg feed ($k_m = 0.7$ was assumed here). For cows with $\Delta W > 0$, MER is corrected with $+19.3 \times \Delta W / 0.65$. FiM applies a final correction of -10 MJ to all MER. T&K predict MER of *ad libitum* fed cows as $0.325 \times W^{3/4} + 19.3 \times \Delta W + 3 \times MY$, divided by efficiency parameter $k = 0.6$. These FiM and T&K equations were used in Minitab to predict the MER of cows that are in energy balance (EB) or with $\Delta W = +0.5$ or -0.5 kg/d and with MY ranging from 5 to 60 kg/d.

Results MER predictions of FiM were a few percent higher than those of T&K at very low and high MY but more similar or slightly lower in the 10 to 40 kg MY range (Figure 1a and 1b). W loss or gain resulted in slightly higher (mean +0.014) or lower (mean -0.017) ratios, respectively, than those depicted in Figure 1b. MER for weight gain were higher (by +1.2 MJ/d) in T&K than in FiM. Reduction in MER as a result of W loss was constant in T&K but in FiM increased with MY to around 48 kg/d and then suddenly decreased for $MY > 50$ kg/d as a result of a discontinuity in the effect of MY on the partial lactational efficiency (i.e. the efficiency of ME utilisation for the NE in the last kg of milk) in the FiM system (Figure 1c).

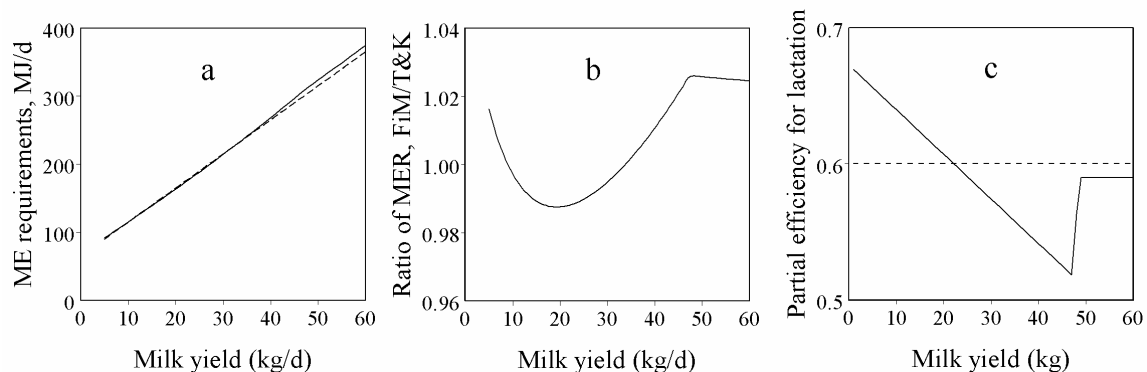


Figure 1 Predicted MER of a cow in EB with $W = 600$ kg by the 13-parameter curvilinear FiM system (solid line) and the 4-parameter linear T&K system (broken line) (a), the ratio of MER as predicted by FiM and T&K (b) and the partial efficiency of ME utilisation for NE in milk in FiM (solid line) and T&K (broken line) (c), all in relation to milk yield.

Conclusions The three parameters in FiM that relate MER for activity to W and feed quality have trivial effects on total MER. The NE_m parameter in FiM is very high compared with T&K and earlier estimates (AFRC 1993). Its effect on effective final ME_m is much reduced, however, as a result of the parameter -10 MJ/d, which leads to internal inconsistencies in the FiM model. The high NE_m estimate was the reason for FiM to fit a curvilinear model to a data set that was essentially linear (Agnew *et al*, 2004). The curvilinearity of the model was constrained by parameter ME_m and the consequences of the curvilinearity for high yielding cows was capped by parameter $k_1 = 0.59$, with very unattractive consequences for partial energetic efficiency (Figure 1c). Despite considerable differences between systems in NE_m estimates, (curvi-) linearity of NE_l response to ME intake and parameter requirements, predicted MER by FiM and T&K are very similar for cows with MY between 10 and 40 kg/d. MER predictions by FiM are slightly higher than those by T&K for cows with very low or very high MY. Data obtained with such cows could provide a critical test which of the two systems predicts MER more accurately. The analysis suggests, however, that the much simpler T&K system gives similar predictions of MER compared to the FiM system while avoiding the inconsistencies of the latter, but could benefit from updated parameter values.

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Milk production and composition and tissue energy balance of early lactation dairy cows fed diets supplemented with calcium salts of fatty acids (Megalac)

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Introduction Supplemental fat is often used to increase the energy density of diets for dairy cows, with the aim of improving tissue energy balance (TEB), milk production and herd fertility. In this study, the effects of supplemental calcium salts of palm fatty acids (CSFA) on production parameters, dry matter intake (DMI) and TEB were assessed in highly productive, early-lactation cows offered typical UK diets.

Materials and methods Multiparous Holstein cows, blocked according to 305-d yield (9663 ±181 kg) in their previous lactation and pre-calving bodyweight (644 ±9 kg), were allocated at calving to either (1) Control - a diet containing no supplemental fat, or (2) CSFA - a similar diet supplemented with Megalac (Volac International Ltd) (n=14/treatment). The study continued until week 17 of lactation. Each treatment was fed *ad libitum* as a TMR containing grass silage, maize silage, cracked wheat, rapeseed meal, soyabean meal and minerals (250, 250, 167, 100, 94 and 8 g/kg DM respectively), with additions of molassed sugar beet pulp, molasses and limestone (96, 32 and 3 g/kg DM respectively) in the Control diet and molassed sugar beet pulp and Megalac (109 and 22 g/kg DM respectively) in the CSFA diet. Respective dietary concentrations (g/kg DM) of crude protein, metabolisable energy (ME; MJ/kg DM), NDF, starch, water soluble carbohydrate and oil were 180, 11.6, 310, 205.78 and 28 for Control and 181, 12.1, 315, 205.59 and 47 for CSFA. Animals were fed individually using Calan gates. Body weight and condition score (5-point scale) were measured twice and once per week respectively. Milk samples for fat, protein and lactose analysis were taken from two consecutive milkings once per week between weeks 2 and 14 of lactation. Progesterone concentration was measured by enzyme-immunoassay (Sauer *et al.*, 1986) in milk samples collected 3 times per week until the cow reached 60 days in milk. Luteal activity was considered to have occurred when milk progesterone concentrations were > 3 ng/ml for 2 consecutive samplings. Blood samples for analysis of β-hydroxybutyrate and non-esterified fatty acids (NEFA) were collected from the tail vein once in weeks 2, 6, 10 and 14 of lactation. Tissue energy balance was calculated as ME intake minus ME requirement (Agnew *et al.*, 2004). The data were analysed as repeated measures within cows using the MIXED procedure of SAS (2003). The following model was tested for each variable: Y = Block + Treatment + Week + Treatment*Week.

Results Treatment did not affect (P>0.05) condition score, bodyweight or DMI (Table 1), but milk yield increased (P<0.05) by 4.3 kg/d overall when CSFA were fed, with significantly higher yields between weeks 7 and 17 (Table 1 and Figure 1). Milk fat and lactose concentrations were unaffected (P>0.05) by treatment, but protein concentration was higher (P<0.05) in the Control group. Yields of fat, protein and lactose, as well as TEB, were similar (P>0.05) across treatments. Significant week by treatment interactions were noted for milk fat (P<0.05) and protein (P<0.001) concentrations, which increased after peak lactation in the Control group but remained constant in the CSFA group, primarily reflecting the diluting effect of greater milk yield in the CSFA animals. The interval from parturition to commencement of luteal activity was similar (P>0.05) in Control (30 d; SEM 3.4) and CSFA (32 d; SEM 3.4) groups. Plasma concentrations of β-hydroxybutyrate (0.839 and 0.828 mmol (SEM 0.07) for Control and CSFA, respectively) and NEFA (0.115 and 0.183 mmol (SEM 0.03) for Control and CSFA, respectively) were similar (P>0.05).

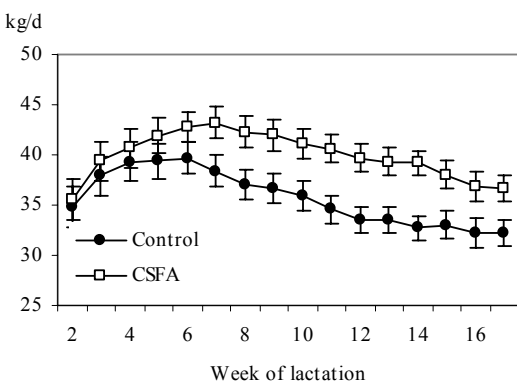


Figure 1 Milk yield (±SEM) during weeks 2-17 of lactation

Conclusion Using Megalac to increase dietary energy density resulted in a significant increase in milk production in early lactation cows. This was achieved without negative effects on DMI, body condition or TEB.

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Table 1 Bodyweight, condition score, dry matter intake, milk yield and composition and estimated tissue energy balance

	Control	CSFA	SEM	Sig.
Condition score	2.07	2.06	0.044	NS
Bodyweight, kg	627	635	17.0	NS
DMI, kg/d	22.6	22.1	0.72	NS
Milk yield, kg/d	35.7	40.0	1.39	*
Yield and concentration of milk components, weeks 2-14 of lactation				
Fat, g/kg	42.4	40.3	1.09	NS
Protein, g/kg	33.4	30.8	0.70	*
Lactose, g/kg	44.6	44.9	0.24	NS
Fat yield, g/d	1537	1621	72.9	NS
Protein yield, g/d	1207	1240	34.3	NS
Lactose yield, g/d	1629	1814	74.8	NS
TEB, MJ/d (weeks 2-14)	-20.5	-25.6	3.27	NS

Effect of grass species on efficiency of nitrogen utilisation in Holstein-Friesian dairy cows

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Introduction Rapid breakdown of herbage proteins in the rumen and inefficient capture of nitrogen (N) by the rumen microbial populations are a major source of N loss and pollution in pasture-based ruminant agriculture. Degree of cell damage during mastication and ingestion varies between grass species with consequences for release of cell contents (protein, sugars and lipids) into the rumen (Kim *et al.*, 2008). Consequently, grazing cattle on different grass species may provide an opportunity to manipulate N efficiency. The purpose of this study was to compare N utilisation efficiency by dairy cattle grazing grass species differing in chemical and morphological characteristics.

Materials and methods Twelve multiparous Holstein-Friesian cows in mid-lactation (with similar milk yields, profiles and stage of pregnancy) were grazed on three forage plots; timothy (TIM; cv. Promesse), perennial ryegrass (PRG; cv. AberDart) or tall fescue (TF; cv. Excella). All animals received 4 kg of dairy concentrate daily. Pairs of cows were allocated to treatment at random in a replicated 3×3 Latin square design. Each measurement period comprised 2 weeks preceded by 3 weeks grazing a standard pasture. At the end of each measurement period chemical composition of the herbage was assessed and herbage intake was estimated using enclosure cages (Lee *et al.*, 2001). Milk yield was recorded throughout the measurement period. Milk samples and spot urine samples were taken during each milking on the final two days. Milk samples were analysed for fat and protein, and N and purine derivatives (PD) were determined in urine. Effects of grass species were examined by analysis of variance with cow pair and period treated as random effects.

Results The N, neutral detergent fibre (NDF) and water-soluble carbohydrate (WSC) contents of TIM, PRG and TF were quite variable between measurement periods (Table 1). Herbage intake estimates were consistently lower for TF.

Table 1 Chemical composition (g/kg DM) of forages and estimated herbage intake (kg DM/d)

	TIM			PRG			TF		
	Period 1	Period 2	Period 3	Period 1	Period 2	Period 3	Period 1	Period 2	Period 3
N	17.2	13.4	25.6	15.2	13.1	23.0	18.7	15.9	22.2
NDF	541	691	520	518	569	492	507	591	567
WSC	156	65	78	342	172	194	186	138	114
DMI	17.7	18.0	19.1	24.5	18.8	16.5	17.3	13.9	13.2

Yields of milk and milk protein were lower ($P < 0.05$) for TF but there was no difference in milk fat output amongst forage treatments (Table 2). Urinary PD:creatinine ratio, an index of rumen microbial protein synthesis, was lower for TF ($P < 0.05$). Relative urinary N output (urine N / creatinine), although not significantly different between forages, was numerically higher for TF.

Table 2 Milk production and N utilisation

	TIM	PRG	TF	SEM [#]	P
Milk yield (kg/d)	22.3	23.3	20.4	0.48	0.005
Milk protein (g/day)	793	796	682	20.8	0.007
Milk fat (g/day)	892	917	883	21.2	0.528
Urinary PD : Creatinine (mmol/L : mmol/L/kg W ^{0.75})	456	439	370	18.1	0.023
Urinary N : Creatinine (g/kg : mmol/L/kg W ^{0.75})	172	176	185	8.9	0.612

[#] ; 8 degrees of freedom for error

Conclusions Tall fescue supported lower milk yield and milk protein production than either PRG or TIM. This effect was associated with lower intake of TF but also reduced microbial protein synthesis in the rumen which may in part be linked to differences in the extent of release of nutrients during mastication and ingestion (Kim *et al.*, 2008). Overall, cows grazing TF pasture showed lower efficiency of N utilisation with approximately 27-40% more urine N per kg of milk protein, despite relatively similar protein content of the different cultivars when averaged across the study.

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Effect of dietary phosphorus level on energy metabolism and phosphorus utilisation in first lactation dairy cows

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Introduction Reducing the phosphorus (P) content of dairy cow diets has the potential to help reduce P losses. However, P is an essential nutrient, and has many roles within the body, while in addition, rumen microbes have a requirement for P (NRC, 2001). Thus, if dietary P levels are inadequate, this could have a negative effect on rumen function, nutrient utilisation and energy metabolism. An experiment was undertaken to examine the effect on energy metabolism and P utilisation of offering dairy cows diets containing reduced levels of dietary P.

Materials and methods Sixteen first lactation Holstein-Friesian dairy cows were allocated to diets containing either 'High (H)' (n = 8) or 'reduced (R)' (n = 8) levels of dietary P, post-calving. Diets offered comprised grass silage and maize silage (approximately 70 : 30 DM basis), supplemented with 10 kg concentrate/cow/day. Total ration P content was adjusted by modifying the amount of P in the concentrate component of the diet, with concentrates formulated to contain either 4.4 or 7.2 g P/kg DM. The concentrate containing the lower P level was formulated using feed ingredients low in P and without additional mineral P being added, while di-calcium P was added to this concentrate to produce the higher P concentrate. On four separate occasions [one (30, s.d., 5.8 days), two (63, s.d., 8.3 days), three (99, s.d., 8.6 days) and five (160, s.d., 7.9 days) months post-calving] these cows were transferred in pairs (one cow from each of the two P treatments) from a group housed situation, and tied in individual stalls within a cow shed. After two days in this environment, each cow was placed in an indirect open-circuit respiration calorimeter for 72-hours. Measurements of gaseous exchange during the final 48-hour period in the chambers were used in the calculation of heat production. On completion of the three-day period in the chambers, animals were returned to individual standings in the cow shed where a six-day ration digestibility study was undertaken. On completion of this, cows were returned to the group housing situation, and continued to be offered their experimental diets. Data was analysed by REML repeated measure analysis of variance, using a correlation model which included cow as a random effect, month post-calving as the time point, and P level as a fixed effect.

Results Dietary P level had no significant effect on either food intake or milk yield ($P > 0.05$), while intakes increased and milk yields decreased ($P < 0.001$) with month post-calving. Dry matter digestibility was unaffected by treatment ($P > 0.05$). Dietary P level had no significant effect on any of the energy utilisation parameters examined, while milk energy/gross energy (GE) intake and heat energy/metabolisable energy (ME) intake, decreased with month post-calving. P intake, P out in faeces, and P balance, were significantly lower with animals offered the reduced P diet ($P \leq 0.01$), while milk and urinary P outputs were unaffected by treatment ($P > 0.05$). P balance increased with month post-calving ($P < 0.001$).

Table 1 Effect of dietary P level on cow performance, and energy and P utilisation

	P level				Month post-calving					
	H	R	SED	Sig.	1	2	3	5	SED	Sig.(lin)
DM intake (kg/day)	17.6	16.8	0.66	NS	14.4	17.5	19.2	17.8	0.75	***
Milk yield (kg/day)	25.9	25.9	0.86	NS	28.0	26.2	26.5	22.9	0.91	***
DM digestibility	0.75	0.75	0.007	NS	0.74	0.75	0.75	0.74	0.010	NS
<i>Energy utilisation</i>										
Energy digestibility	0.74	0.74	0.006	NS	0.73	0.74	0.74	0.75	0.006	NS
Methane E/GE intake	0.068	0.070	0.0026	NS	0.066	0.070	0.068	0.072	0.0028	NS
Urine E/GE intake	0.029	0.028	0.0015	NS	0.028	0.030	0.027	0.029	0.0034	NS
Milk E/GE intake	0.26	0.26	0.009	NS	0.32	0.26	0.24	0.22	0.011	***
Heat E/ME intake	0.64	0.67	0.0185	NS	0.70	0.66	0.63	0.63	0.0284	*
Retained E /ME intake	-0.04	-0.08	0.024	NS	-0.21	-0.06	0.0	0.02	0.041	***
kl	0.57	0.54	0.017	NS	0.55	0.54	0.56	0.56	0.025	NS
<i>Phosphorus utilisation</i>										
P intake (g/day)	96	71	3.3	***	71	86	92	85	3.49	***
Faecal P out (g/day)	58	42	2.1	***	46	50	53	52	2.60	*
P apparent digestibility	0.39	0.40	0.020	NS	0.35	0.42	0.43	0.40	0.023	*
Urinary P out (g/day)	0.6	0.1	0.29	NS	0.7	0.5	0.2	0.1	0.62	NS
Milk P out (g/day)	26	25	1.2	NS	27	25	26	24	1.0	***
P balance (g/day)	11.5	4.2	2.6	*	-2.3	11.0	13.0	9.7	2.62	***

Conclusions Dietary P level had no effect on feed intake, milk output, or on any of the energy utilisation parameters examined, thus suggesting that neither rumen function, nor tissue metabolism, were affected by P level. However, reducing dietary P levels resulted in a 28% reduction in faecal P output, thus highlighting the potential of this practice to reduce P excretion by dairy cows, and as such reduce the risk of P entering watercourses.

Acknowledgements Funded by DARDNI, AgriSearch, John Thompsons and Sons Ltd and Devenish Nutrition Ltd

Reference National Research Council (NRC) (2001) : Nutrient Requirements of Dairy Cattle, Seventh Revised Edition, National Academy Press, Washington DC.

Effects of level and form of dietary zinc on apparent absorption and retention in dairy cattle

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Introduction There has been growing public concern with regard to the effects of heavy metals excreted by animals on the environment. Heavy metals can be lost from manure and slurry to ground and surface waters, and subsequently affect water quality (Van Horn *et al.*, 1996). In regions of high domestic livestock production, farmers are therefore seeking means to reduce the quantity of heavy metals excreted by animals and subsequently onto the land (Van Horn *et al.*, 1996). The objective of this study was to establish the effect of two levels of dietary Zn inclusion in two different forms (organically chelated *vs.* inorganic) on apparent absorption and retention in dairy cattle.

Materials and methods A basal ration containing (DM basis, kg/kg) 0.39 maize silage, 0.19 grass silage, 0.08 urea treated wheat, 0.05 soyabean meal, 0.05 rapeseed meal, 0.07 molassed sugar beet pulp, 0.02 maize gluten feed, 0.05 molasses, 0.01 fat, 0.01 minerals (without zinc) and 0.08 concentrates was offered at 1.05 of *ad libitum* intake. The basal diet was predicted to supply 81 mg of Zn per day and was supplemented with one of four concentrates differing in their level and form of dietary Zn. The concentrates provided an additional 600 mg Zn/d (to supply the total recommended level (NRC (2001)); R) or 120 mg Zn/d (to supply 0.7 of the total recommended level; L), either supplemented as ZnO (I) or organically bound Zn (O) (Bioplex ZnTM; Alltech Inc., Nicholasville, USA) and were fed at the rate of 2 kg/cow/day in one meal. Four Holstein-Friesian dairy cows producing on average 31.1 kg (s.d. +/-3.7) of milk per day were used in a 16 week study. The cows commenced the study in their 8th week of lactation. There were four periods, each lasting 28 days in total with a 21 day adaptation to the diets, including 2 days adaptation to the metabolism room and 7 days total collection of faeces and urine. During the final 7 days of each period, milk yield was recorded daily with samples taken on days 4 and 5 for subsequent analysis of milk Zn. Blood samples were taken via venepuncture from the coccygeal vein into lithium heparin tubes on day 7 of the sampling period. All samples were analysed by Inductively Coupled Plasma Mass Spectrometry. The data were analysed by analysis of variance as a 4 x 4 Latin square design. Treatment degrees of freedom were split into main effects of Zn level (ZnL), form (F) and their interaction (ZnLxF). All statistical analysis was conducted using GenStat version 10 (VSN Int. Ltd., Oxford, UK).

Results Dry matter intake averaged 22.6 kg/d and milk yield averaged 30.7 kg/d but neither differed between treatments ($P>0.05$). Intake of Zn (mg/d) was lower ($P<0.001$) for cows receiving the low level of Zn (L) compared to the recommended level (R), with the subsequent amount of faecal Zn also being lower ($P<0.001$). Cows receiving O had lower urine Zn (mg/d) ($P<0.05$) compared with cows receiving I. Milk Zn did not differ ($P>0.05$) between treatments. Overall daily Zn retention (daily Zn intake – (faecal Zn+ milk Zn+ urine Zn)) for cows on RI, RO or LO resulted in positive retention, whereas cattle fed the LI treatment had a negative retention of -19 mg/d Zn. Plasma Zn was similar between treatments, with a mean of 12.3 µmol/l.

Table 1 Effect of level and form of dietary zinc on dairy cow performance and apparent absorption and retention.

	Treatments				Significance (P)			
	RI	RO	LI	LO	s.e.m.	ZnL	F	ZnLxF
Total DMI (kg/d)	22.0	21.5	23.3	23.3	0.90	0.146	0.838	0.783
Milk yield (kg/d)	29.6	30.2	31.2	31.9	1.21	0.227	0.624	0.995
Diet Zn (mg/kg DM)	62.4	65.3	39.7	39.7				
Zn (mg/d)								
Intake	1372	1405	925	924	30.0	<.001	0.632	0.595
Faecal	1115	1156	811	742	41.3	<.001	0.742	0.243
Urine	0.95	0.90	1.05	0.85	0.069	0.600	0.047	0.194
Milk	133	120	132	140	10.6	0.398	0.848	0.376
Apparent absorption	257	249	114	181	48.2	0.081	0.565	0.471
Apparent absorbed Zn (g/g)	0.19	0.17	0.12	0.20	0.046	0.585	0.397	0.224
Zn retention (mg/d)	123	128	-19	40	43.3	0.045	0.495	0.555
Plasma zinc, µmol/l	12.1	11.6	12.6	12.7	0.88	0.416	0.838	0.707

Conclusions. Increasing dietary Zn level increased the amount of Zn apparently absorbed and retained but also increased the amount excreted. Organically chelated Zn resulted in a lower urinary Zn output but did not affect overall apparent absorption or retention.

Acknowledgements Financial support of Alltech (UK) Ltd and DEFRA is gratefully acknowledged.

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Effects of an oral supplementation of lactoferrin or of the association lactoferrin-lactoperoxidase system to newborn calves on health and production parameters

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Introduction Neonatal diarrhoeas are a major problem in bovine husbandry. Antibiotics are usually utilized as medicine but a prevention based on natural methods could be of interest. Lactoferrin (Lf) is a glycoprotein naturally found in milk. It is known for its many useful properties for animal health, in particular for its immunomodulator, antimicrobial, anti-inflammatory and antiviral effects. The lactoperoxidase system (LpS), an enzymatic system, also present in milk, possesses effective antimicrobial properties. It is composed of lactoperoxidase (Lp), thiocyanate and H₂O₂. A synergy between Lf and LpS has been reported. The aim of this study was to assess the effects of Lf and of the association Lf-LpS as oral supplements to newborn dairy calves in order to improve health and production parameters.

Materials and methods Thirty six Holstein calves from the Experimental Station of the University of Liege were allocated, at birth, to one of three groups: "control" which was not supplemented, "Lf" which received 1g of Lf per day and "Lf-LpS" which was supplemented once daily with the association Lf-LpS (0.18g Lf, 0.05g Lp, 0.23g potassium thiocyanate, 0.03g glucose oxidase, 5.52g glucose). The experiment was performed during the first 3 weeks of the life. Calves received at birth 0.5l of lyophilised colostrum and 12h later, 0.5l of a mix of colostrum from 3 different cows of the herd. From day 1 to day 21, calves were fed twice daily with a milk replacer and had free access to water, hay and calf starter. Calves were weighed and faecal consistency scores (0 = firm, 3 = aqueous diarrhoea) were evaluated twice weekly. The coliforms count in faeces was determined at days 2, 7 and 14. The number of days of diarrhoea and the medication days necessary to eradicate these diarrhoeas were recorded. Blood samples were taken at days 7 and 14. The data obtained on different days were statistically analysed by use of a mixed model including the effects of supplementation, time and sex with an autoregressive covariance structure of type 1. Contrasts were used to compare the parameters changes in different groups relative to base line. The single data were analysed with a chi-square test.

Results Both supplements tended to reduce the frequency of diarrhoea (0.45 and 0.50 versus 0.62 in the Lf, Lf-LpS and control groups respectively). The medication days and the faecal consistency scores tended also to be reduced. The present results with Lf supplement were in agreement with those of Robblee *et al.* (2003). In the control group, the coliforms count did not change according to the time (Figure 1). By contrast, there was a large and significant (P<0.05) reduction in the coliforms count in the Lf-LpS group. Surprisingly, there were no differences in coliforms counts between Lf and control groups. The live weight decreased in the control group up to the second week and then, started to increase (Figure 2). The pattern of the decrease was much reduced in the supplemented regimes, the differences in live weight being significant at day 7 (P<0.01) and at day 10 (P<0.05) but with no differences between supplements. The average iron plasma concentration was 15.0 µmol/l in the Lf-LpS group while it was 11.2 and 10.9µmol/l in the control and Lf groups (P=0.06). Similar differences between calves groups were observed for red cells counts, hematocrit and hemoglobin content. By contrast, the supplementations did not affect white cells counts and the Lf content in blood serum.

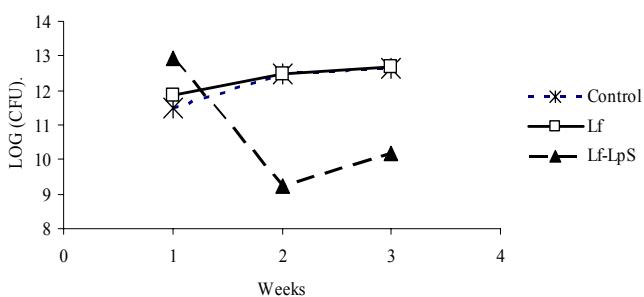


Figure 1 Changes in faecal coliforms counts

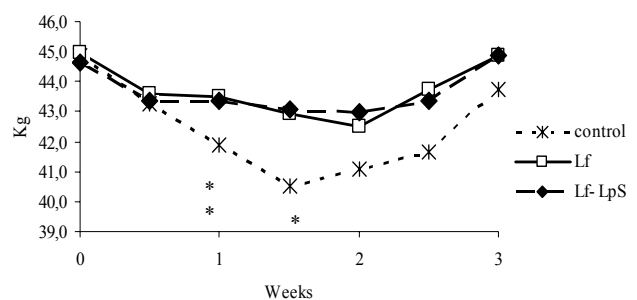


Figure 2 Changes in body weight ** (P<0.01), * (P<0.05)

Conclusions The positive effects of Lf and Lf-LpS supplementations to newborn dairy calves are of interest not only in terms of animal production but also in terms of an animal welfare point of view owing to use of natural products rather than antibiotics

Acknowledgements Financial support was provided by DGA - Region Wallonne - Belgium.

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The effect of over wintering strategies on performance and carcass characteristics for cull dairy cows on a subsequent grass based finishing strategy

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Introduction Cull cows comprise about 44% of all cattle slaughtered at Irish meat factories in 2006; which was an increase of 6% from 2005. Between September and December 2006, 18% of cull cows failed to achieve P+3 carcass classification compared with 12% for the remainder of the year (DAF, 2006). There is a large proportion of cows slaughtered in November, which suggests that unfit (low bodyweight and condition score) cows are being presented for slaughter at the end of lactation without finishing prior to slaughter. Farmers who are finish feeding cull cows have an interest in alternative feeding regimes i.e. pasture and/or forage use for a more economical beef supply due to increasing concentrate costs, however present farm practice and culling decisions made by the dairy farmer often excludes this as a real possibility. The objective of this study was to compare days to slaughter, average daily gain, and final live and carcass of cull dairy cows subjected to four over-wintering strategies prior to a pasture based finishing diet.

Materials and methods The experiment was undertaken at Teagasc, Moorepark from 18 December 2006 to 29 June 2007. Fifty-six Holstein-Friesian cull dairy cows were randomised across a four treatment (n=14) finishing experiment. They were randomised on age, 68 (s.d. 25.58) months, lactation number, 3.5 (s.d. 2.08), Holstein proportion, 0.75 (s.d. 0.25), liveweight, 607 (s.d.70.0) kg and body condition score, 2.75 (s.d. 0.38). The treatments were; slaughtered on Day 0 (C), 0.70 grass silage and 0.30 straw (GS+SW), grass silage offered *ad-libitum* (GS), milking treatment offered *ad-libitum* grass silage plus 6 (kg) concentrate DM/cow/day (MGS+6C). Dietary treatments were group-housed according to individual treatment in a lime dusted concrete cubicle house. Grass silage was 0.32 (s.d. 5.15) DM and 0.70 DMD. Finishing criteria applied was similar to Minchin *et al.*, (2007). The criteria were; carcass weight > 272 kg, fat score 3 and carcass classification P+ or O. Liveweight, BCS, back fat, milk yield, group intake and carcass characteristics were measured. Individual DM intake was measured twice, at weeks 7 (indoor) and 15 (grazing) using the n-alkane technique. Following a 12 week wintering period (P1) cows were turned out to grass (P2). They were managed as a single herd and offered *ad libitum* herbage, grazing to 5cm post grazing height. Post-slaughter measurements included carcass classification and carcass fabrication. All statistical analyses were performed using SAS. All individual animal variables were analyzed (n=56) using analysis of variance.

Results Table 1 shows the results of physical, carcass and feed intake performance for the four treatments. The MGS+6C treatment yielded 16.4 kg (s.d. 3.51) milk/cow/day, 40.9 g/kg (s.d. 5.59) fat, 35.0 g/kg (s.d. 3.00) protein and 43.6 g/kg (s.d. 1.26) lactose over a milking period of 77 days. As the finishing criteria were predetermined, there was no significant effect between the three finishing treatments on slaughter live and cold carcass weight; however there were significant differences between the control treatment and finishing treatments. Slaughter BCS was significantly different (P<0.05) between treatments. Cows on the GS treatment (115 days) finished on average 33 and 38 days earlier than the GS+SW and MGS+6C treatments. Kill out proportion was similar (0.46%) across dietary treatments. The GS+SW, GS and MGS+6C treatments achieved an ADG of 0.76, 0.87 and 0.45 kg, respectively from trial start to slaughter. Pre-grazing sward height was 14.3 (s.d. 5.54) cm/day, daily herbage mass was 2812 (s.d. 1683.9) kg DM/ha with daily herbage allowance in P2 of 15.2 (s.d 5.31) kg DM/cow/day. Post-grazing sward height was 5.2 (s.d. 1.26) cm/day, with 90% (s.d. 16.0) utilization; herbage removal was 13.5 (s.d. 4.91) kg DM/cow/day.

Table 1 Mean values of performance characteristics for cull dairy cows offered a grass based finishing diet

Treatment	Day 0	GS+SW	GS	MGS+6C	Sed	Sig
Slaughter Liveweight (kg)	599 ^a	714 ^b	695 ^b	685 ^b	17.02	***
Slaughter BCS	2.79 ^a	3.52 ^b	3.59 ^c	3.50 ^b	0.04	*
Weight gain (kg/day)	N/A	0.76 ^a	0.87 ^a	0.45 ^b	0.15	*
Period on trial (days)	N/A	148 ^a	115 ^b	153 ^a	12.06	**
Carcass cold weight (kg)	251 ^a	329 ^b	322 ^b	319 ^b	8.38	***

*P<0.05; **P<0.01; ***P<0.001. ^{abc}Means, in rows, with a different superscript differ significantly. N/A, non applicable

Conclusions The results show it is possible to dry off and finish feed cull cows at pasture regardless of over-wintering strategy. The GS treatment was the most efficient treatment evaluated, this was a result of high ADG with feeding of *ad libitum* grass silage followed by finishing at grass. The GS+SW and the MGS+6C treatments achieved satisfactory performance, however over-winter feed restriction and milk production created prolonged finishing periods for both treatments. Pasture based strategies will significantly add to cull cow carcass value.

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Effect of the inclusion of a live yeast culture into mixed forage diets on milk yield, locomotion score, lameness and sole bruising in first lactation Holstein Friesian dairy cattle

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Introduction Yeast has been used as a means of manipulating rumen fermentation to improve production performance because of the effect on stimulating cellulolytic micro-organisms in the rumen that increase fibre digestion, alter acetate to propionate ratios and increase microbial protein flow to the duodenum (Wallace and Newbold, 1992). Williams *et al.* (1991) found that animals offered high energy diets had decreased rumen lactic acid concentrations, which is associated with higher rumen pH and characteristic of more stable rumen fermentation. Low rumen pH has been associated with poorer hoof quality (Westwood *et al.*, 2003). This research aimed to assess the effect of the inclusion of live yeast in total mixed rations on milk yield, lameness, sole bruising and hoof characteristics.

Materials and method The heifers (34) were allocated into one of two groups at parturition between 30 November and the 31 December 2005 and allocated in pairs according to breeding value, body condition score and live weight. The heifers were all offered *ad libitum* access to the same total mixed ration (TMR) for a total of 114 days, which was either with the addition of live yeast at the rate of 10 billion/h/d (Yeast) or with no additional live yeast (No yeast). Locomotion score was assessed weekly using a scoring system with a 5 point scale; 1 non lame, 5 lame (Tranter and Morris, 1991). Claws were assessed for sole ulcers, sole haemorrhage and heel erosion at 50, 100, and 150 d *postpartum*. Other alterations present during examination, such as *Digital dermatitis* and heel horn erosion were recorded. The lesions on each foot were scored according to Leach *et al.* (1998). The affected areas of the sole and white line areas of the cow's foot were marked and subsequently photographed with a digital camera and the images were analysed for the size of claw area, white line and haemorrhage using Image Analysis program (Scion Image Analysis, 2001) and the white line and sole areas of the hoof were scored separately. The hoof growth and wear rates were measured on the right rear inner claw at 50, 100, and 150 DIM (according to Clark and Rakes, 1982) and the hoof angle, length of the front hoof wall and height of the heel were measured at the same time of the hoof growth and wear, according to Boelling and Pollott (1998). The experiment used individual heifers as observations and inclusion of yeast was included in the model. The hoof measurements were found to be normally distributed and analysed making comparisons by observation period, using analysis of variance (ANOVA), general linear modelling command (Minitab 14.0). Due to the nature of locomotion scores and the number of low values was high and skewed the distribution towards lower values and a non parametric analysis, Mann-Whiney U test (Minitab 14.0) was used.

Results The mean milk yield was significantly higher, while the mean number, percent and total score for sole haemorrhages was significantly lower for cattle offered yeast culture compared with cows not offered the yeast culture. The mean locomotion score and hoof measurements were not significantly different between animals offered and not offered yeast.

Table 1 Mean milk yield and hoof assessment of animals offered mixed diets with or without additional yeast culture

	Yeast	No Yeast	SEM	P
Milk yield (kg/d)	29.5	27.1	0.29	**
Locomotion score (1 to 5)	1.25	1.18	-	NS
Mean number of sole haemorrhages	2.3	3.2	0.28	**
Sole haemorrhage (%)	10.1	18.1	2.13	**
Total score sole haemorrhage (%)	11.5	23.2	2.96	**
White line damage (%)	18.4	17.2	2.95	NS
Total score white line damage (%)	25.6	21.6	4.82	NS
Foot angle (mm)	48.6	49.3	0.34	NS
Dorsal border (mm)	83.6	84.5	1.78	NS
Heel depth (mm)	36.9	35.9	1.08	NS
Diagonal measurement (mm)	116.8	115.8	1.29	NS
Monthly growth (mm)	6.2	6.3	0.98	NS
Monthly wear (mm)	4.5	10.7	2.19	NS

Conclusions The addition of yeast culture to the diet increased milk yield and reduced the number of sole haemorrhages, sole haemorrhage scores. Locomotion score and all other measurements were not significantly different between animals that were offered a mixed ration with or without the addition of a yeast culture.

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Relationships between blood metabolites and hormones before and after first calving in dairy heifers and their fertility during the first lactation

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Introduction A short herd lifespan is a significant economic loss to the dairy industry, with infertility a major cause. Conception rates to first service have fallen from around 60% in the 1970s to 40% by 2000. The re-establishment of ovarian cyclicity after calving is important in determining the timing of conception which should be around 80 days postpartum (PP) to maintain yearly calving intervals. Insulin-like growth factor-I (IGF-I) is thought to influence ovarian activity at this time. We have previously shown that pre-pubertal IGF-I levels are related to growth and can predict concentrations around first calving. This study investigated IGF-I, insulin, glucose, urea and betahydroxybutyrate (BHB) concentrations before and after first calving in typical UK herds in relation to measures of fertility during the first lactation.

Methods A total of 17 dairy farms across southern England milking Holstein-Friesian cows were recruited during 2003 and 2004, providing a range of management practices representative of those commonly encountered in the UK. Heifers that calved for the first time (mean age 25.8 months) were sampled at approximately 1 to 2 weeks before calving and at weeks 1 and 8 PP. Body condition score (BCS) was recorded, and blood samples were analysed for IGF-I, insulin, glucose, urea and BHB. Measures of fertility were recorded after first calving; days to commencement of luteal activity (CLA), days to first service (DFS), days to conception (DTC), services per conception (S/C) and calving interval (number of days between 1st and 2nd calving) (CI). The number of heifers failing to conceive (FTC) during their first lactation was recorded. Animals were subdivided on the basis of their fertility into (i) those that conceived or FTC despite services and (ii) for those that did conceive, if this were before or after 80 days PP. Independent samples t-tests were used to compare the fertility parameters and metabolite concentrations between these groups. All data were tested for homogeneity of variance and log transformed if necessary.

Results Blood samples were collected from 326 heifers calving for the first time. After calving 28 heifers died or were culled or sold before first service PP, and 19 animals were served (1-8 times) but FTC (infertility n=11, other reasons n=8). Fertility data for 279 animals that successfully conceived during their first lactation are summarised in Table 1. Mean pregnancy rate to first service was 47% (130/279).

Table 1 Measures of fertility (n=279)

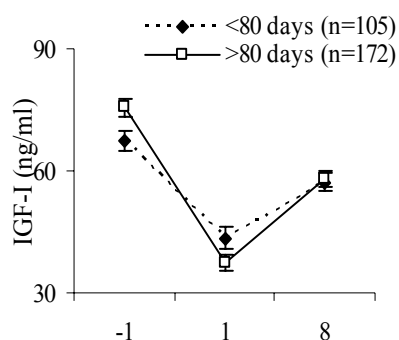
	Mean ± SD	Range
CLA	27 ± 13	7 - 76
DFS	78 ± 28	37 - 297
DTC	113 ± 64	42 - 441
S/C	2.1 ± 1.6	1 - 10
CI	384 ± 60	318 - 718

Table 2 Conceived before or after 80d. Within rows; a<b, P<0.001
*n=277 due to incomplete service records at farm level

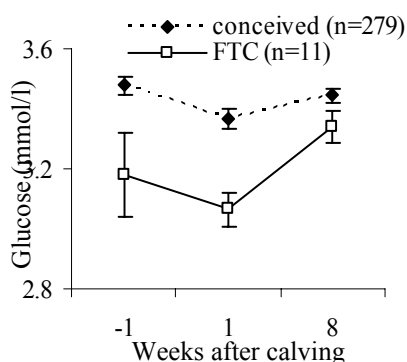
	< 80 days	> 80 days
n*	105	172
CLA	26 ± 13	27 ± 14
DFS	61 ± 10 ^a	88 ± 31 ^b
DTC	65 ± 9 ^a	143 ± 64 ^b
S/C	1.2 ± 0.4 ^a	2.7 ± 1.7 ^b
CI	345 ± 11 ^a	414 ± 63 ^b

The CLA was the same for animals conceiving before and after 80 days PP; however DTC was doubled for animals conceiving after 80 days (Table 2). Animals conceiving at >80 days had significantly higher IGF-I and urea before calving ($P<0.05$), and tended to have lower IGF-I levels at week 1 PP ($P=0.07$) (Figure 1a). Concentrations of insulin, glucose and BHB did not differ between the animals conceiving again before or after 80 days. Animals FTC tended to have higher levels of IGF-I and BHB before calving ($P<0.1$), together with higher insulin and urea concentrations ($P<0.01$) and lower glucose levels ($P<0.05$) (Figure 1b). After calving IGF-I and glucose were lower at 1 week PP for animals FTC ($P<0.05$), and BCS was significantly lower at weeks 1 and 8 PP for animals FTC ($P<0.05$) (Figure 1c). The patterns in IGF-I and urea around calving were therefore similar in the >80 days and FTC groups.

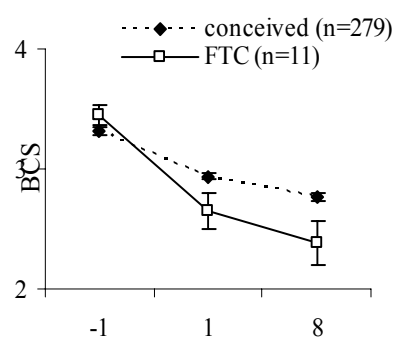
(1a) IGF-I; conceived before or after 80d



(1b) Glucose; conceived versus FTC



(1c) BCS; conceived versus FTC



Conclusions In this study 14% of heifers failed to calve for a second time, with infertility accounting for 23% of disposals during the first lactation. CLA length was the same for all animals, regardless of the time of conception after first calving. Cows at risk of poor fertility after calving could be identified from metabolite concentrations before calving, highlighting the need for good nutritional management of late pregnant heifers.

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The effect of level of milk and plant extracts on growth rate and development of New Zealand Friesian and Jersey Cross Friesian dairy heifers

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Introduction. Calving dairy heifers at 2 years of age, with the achievement of high levels of maturity (live weight) at service (65%) and calving (85 %) increases lifetime productivity (Margerison and Downey, 2005). Recent research into dairy heifer nutrition has focused on increasing calf development, live weight gain and development of stature, while minimising tissue fat deposition, particularly in mammary parenchyma (Serjrsen, 2005). The use of increasing feeding levels and growth rates for dairy heifers during the prepubertal period have been the focus of recent research and much of this has involved differing sources and levels of energy and protein (Hill, 2005; Tannan, 2005) and the use of probiotics and natural plant extracts to replace antimicrobials. In addition to these considerations, the world milk supply dynamics and increasing global demand for milk results in the need to consider using lower levels of whole milk and alternatives to skim milk products for dairy heifer rearing. The aim of this research was to compare the effect of offering differing levels of whole milk and a combination of plant extracts on the growth, development and weaning age of New Zealand (NZ) Holstein Friesian and NZ Holstein Friesian dairy heifers.

Materials and methods 60 calves (NZ Friesian Holstein (FH), NZ Friesian Holstein X Jersey (HF X J) were selected at random from the Massey University Dairy units (NZ) and allocated (48 h of age) according to birth date, breed, stature and live weight to one of three treatments; 4 l/h/d of whole milk (M), 4 l/h/d of whole milk, plus 200 g plant extracts (M+P) 2 l/h/d of whole milk, plus 200 g plant extracts (0.5M+P). All calves had individual feed intake (straw and starter diet) recorded daily and had live weight, height at withers and hip, hip width and girth at the heart and last-rib measured weekly. Calves were weaned at set target weights (kg) according to breed from milk (FH X J: 75 kg; FH: 90 kg) and limited to 2 kg of supplementary feed (FH X J: 80; FH: 100 kg) at pasture. Data was found to be normally distributed and analysed by ANOVA GLM (Minitab 14.0) and regression using individual animals as observations and diet, breed were included in the model. Significant differences were assessed, with a confidence interval of 95%, using Tukey's test.

Results Calves offered 4 l milk and plant extracts had significantly higher live weight gains, higher hip width and hip height and subsequently weaned significantly sooner than calves offered 4 l of milk. All calves offered plant extracts had significantly higher last rib widths. The calves offered 2 l of milk and plant extracts had similar growth and development rates compared with calves offered 4 l milk/h/d.

Table 1 Mean growth rates weaning weights and length of milk and supplementary feeding periods for calves offered differing levels of milk and plant extracts

	M	M+P	0.5M+P	SEM	P
Mean live weight at weaning from milk (kg)	86.6	85.4	84.2	1.68	0.604
Mean milk feeding period (d)	80.2 ^a	72.1 ^b	73.4 ^{ab}	2.34	0.041
Mean milk feeding period mean weight gain (g/d)	648 ^c	757 ^{ab}	729 ^{bc}	17.0	0.034
Mean supplement feeding period to weaning weight (d)	87.3 ^a	79.0 ^b	82.5 ^a	1.10	0.050
Mean supplement feeding period mean weight gain (g/d)	1253	1237	1190	70.3	0.809
Mean weaning weight from adlib supplement (kg)	99.0	97.8	97.1	1.03	0.337

Table 2 Mean wither and hip height, hip width and girth at last rib and heart of calves offered differing levels of milk and plant extracts (0 to 14 weeks of age)

	M	M+B	0.5 M+B	SEM	P
Mean withers height (m)	0.77	0.78	0.77	0.014	0.086
Mean hip height (m)	0.81 ^b	0.83 ^a	0.82 ^{ab}	0.043	0.005
Mean hip width (mm)	18.7 ^b	19.5 ^a	19.0 ^b	0.17	0.004
Mean heart girth (mm)	90.3	92.6	91.6	0.67	0.057
Mean last rib girth (mm)	95.0 ^b	99.4 ^a	99.1 ^a	1.05	0.036

Conclusions Calves offered milk (4l) with plant extracts had significantly higher live weight gains, higher hip width and hip height and subsequently weaned significantly sooner compared with calves offered 4 l of milk with no plant extracts. Calves offered lower levels of milk (2 l) and plant extracts had similar growth and development rates to calves offered 4 l milk with no plant extracts. Offering calves milk with plant extracts increased growth and development rates and reduced the time to target weaning weight.

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Validation of the sulphur hexafluoride (SF₆) tracer technique as a method of predicting methane output from dairy cows

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Introduction In order to assess the impact of methane (CH₄) production from ruminants on the environment, and to measure the effects of various mitigation strategies for methanogenesis, it is necessary to accurately measure CH₄ output under different scenarios. Techniques which enable measurement of CH₄ output from individual animals can facilitate a meaningful investigation of factors affecting methanogenesis. Indirect open-circuit respiration calorimetry chambers are an efficient way to measure CH₄ production from individual animals. However, chamber measurements cannot be used to measure CH₄ output from grazing animals. To overcome this, the sulphur hexafluoride (SF₆) tracer technique was developed by Johnson *et al.* (1994), and is widely used to measure CH₄ emissions from individual grazing ruminants. Although the SF₆ technique has been used quite extensively, relatively few studies have been carried out to validate its accuracy compared to respiration calorimetry studies. Additional validation studies are required to clarify the situation, especially for dairy cows which are major producers of CH₄. Consequently, the objective of this experiment was to use indirect open-circuit respiration calorimetry chambers to validate the SF₆ technique for measuring CH₄ emissions from dairy cows, and to develop a prediction model for actual CH₄ production of dairy cows from SF₆ estimates.

Materials and methods Eighteen Holstein-Friesian dairy cows were used in this experiment: 12 first lactation dairy cows with a mean live weight of 459 (s.d., 50.0) kg at the start of the experiment and 6 multiparous cows with a mean liveweight of 632 (s.d., 86.0) kg. Animals were taken in pairs and placed into individual stalls where they were offered their individual diets. All cows were offered *ad libitum* mixed diets of grass silage with varying amounts of concentrates. Intakes and refusals were weighed and recorded. After three days, each pair of cows was taken to indirect open-circuit respiration calorimetry chambers for four days, with CH₄ output being measured both by the chambers and by the SF₆ tracer technique for the last three days. The SF₆ apparatus was placed in the back of each chamber, with the nosepiece taped inside an air duct through which air was sampled to the gas analysers. A t-test was performed to investigate the difference between CH₄ values estimated from the chamber versus the SF₆ technique. Linear and curvilinear regression techniques were used to develop a prediction equation for actual CH₄ output from SF₆ estimates.

Results Methane output measured in the chambers ranged from 330 to 762 l/d (mean = 489 l/d, s.d. = 130.1) whereas CH₄ output measured by the SF₆ technique ranged from 236 to 565 l/d (mean = 388 l/d, s.d. = 99.7). Methane measured using the SF₆ technique ranged from 61 to 96% of that measured using the chambers (mean = 75%). There was a significant difference ($P < 0.001$) in mean CH₄ output between the two techniques. The lower CH₄ estimate from the SF₆ technique is in line with the results published elsewhere with sheep (Leuning *et al.*, 1999) and beef cattle (Johnson *et al.*, 1994). The correlation between CH₄ output measured using the SF₆ technique and that measured with the chambers is displayed graphically in Figure 1. The equation of the linear trendline is $y = 1.099$ (s.e. 0.1340) $x + 95$ (s.e. 53.1) ($R^2 = 0.76$, $P < 0.001$) where y = chamber methane and x = SF₆ methane. The relationship between CH₄ estimates using both techniques was also examined using a range of curvilinear models, but none of them fitted the dataset significantly better than the linear relationship.

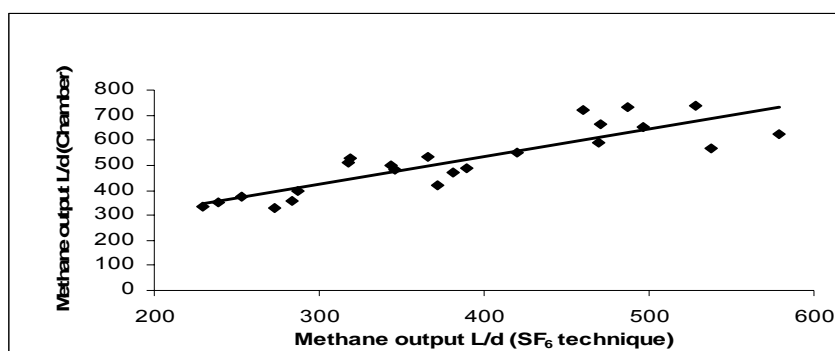


Figure 1 The relationship between CH₄ output as measured by chambers (y-axis) and SF₆ technique (x-axis).

Conclusion A good correlation was obtained between CH₄ output data using the SF₆ tracer technique and that obtained using indirect open-circuit respiration calorimetry chambers.

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Effects of microbial inoculants on the dry matter intake, nutrients digestibility and chewing activity and performance of Iranian Holstein Dairy cows

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Introduction Lactic acid bacteria (LAB) are often used as an inoculant to control the ensiling fermentation by rapid production of lactic acid and the consequent decrease in pH and improved silage preservation with minimal fermentation losses and fermentation of grass and legume silages. Microbial inoculation appears to have minimal effects on the fermentation of corn silage (Bolsen *et al.*, 1992). However, changes in silage fermentation have not always been related to improved animal performance. However, Wohlt (1989) reported that inoculated silages appeared to be more stable upon exposure to air and, when fed to cows, increased FCM by 0.7 kg/d. The objective of this study was to determine the effect of microbial inoculation on the fermentation of corn silage treated with bacterial inoculants and the subsequent effect on nutritive value and performance by lactating dairy cattle.

Material And methods Whole plant corn with 24.2 % DM was harvested and chopped by a conventional forage harvester and was ensiled in two big horizontal silos that had capacity more than 50 tones. Silages were prepared without inoculants (control) or with one of commercially available inoculants (*LALSIL MS01*, *LALLEMAND*, FRANCE). After 30d of ensiling, each silo was opened, sampled and their pH was measured directly on the silage juice, using a pH meter. Six lactating Holstein dairy cows (BW=589/5 ± 43/84) were allotted to 2×2 repeated Latin square design at two 21d periods (adaptation, 14 d, and sample collection, 7 d) to evaluate the effects of a new microbial inoculant product on the dry matter intake, nutrients digestibility and chewing activity and performance of Holstein Dairy cows. Two treatments were contained 1) basal ration + untreated corn silage; 2) basal ration + treated corn silage with microbial inoculant (MS01 Lalsil, LALLEMAND that contained 3*10¹⁰(cfu), 3*10¹⁰(cfu) and 1 (g) of *Lactobacillus plantarum*, *Propionibacterium acidipropionici* and glucose). Samples of silage, rations, and faeces were dried at 55 °C for 48 h in a forced-air oven for DM, ground through a Wiley mill (1-mm screen pore size), analyzed for DM, CP, EE, ash, NDF. Non fibrous carbohydrate was calculated by 100- (%CP+ %NDF+ %Ash+ %EE). The concentration of N-NH₃ was measured with Kjeltec Auto Analyzer.

Results and discussion The results are presented in Table 1. Inoculation of silage significantly reduced pH of silage and concentrations of ammonia-N of corn silages compared with the control silage. The NDF content of inoculated silage was significantly lower than control silage which is result of more fermentation of NDF content of silage in inoculated silage. In addition, the lower NDF content of inoculated silage is the result of partial acid hydrolysis of hemicelluloses (Filya, 2004). The inoculant significantly increased daily dry matter intake (18.17 vs. 22.18 kg/day) and subsequently NDF (11.27 vs. 13.08 kg/day), ether extract (1.09 vs. 1.55 kg/day), crude protein (2.29 vs. 2.86 kg/day) and ash 2.54 vs. 3.32 kg/day; eating (258.33 vs. 295.83 min/day) and rumination time (452.50 vs. 488.33 min/day), total chewing activity (700.83 vs. 784.17 min/day), milk yield (21.00 vs. 22.54 kg/day), without significant effect on apparent digestibility of DM and nutrients, passage rate of solid material, ruminal mean retention time, percentage of milk fat and protein.

Table1 Effect of microbial inoculants on intake and nutrients digestibility of corn silage-based diets fed to lactating cows. (Means within a row with different superscripts differ (P<0.05)).

item	Treatments		SEM	P-Value
	uninoculated	inoculated		
Dry matter intake (kg/d)	18.17 ^b	22.18 ^a	0.186	<0.0001
Digestibility of dry matter (%)	68.85	69.23	0.790	0.7373
Digestibility of NDF (%)	64.21	62.83	0.970	0.3224
Digestibility of ash (%)	76.27 ^b	78.76 ^a	0.530	0.0025
Eating time (min/day)	258.33 ^b	295.83 ^a	0.223	0.0001
Rumination time (min/day)	452.50 ^b	488.33 ^a	0.276	0.0003
Total chewing activity (min/day)	700.83 ^b	784.17 ^a	0.456	<0.0001
Particulate passage rate (%/h)	3.41 ^b	3.53 ^a	0.015	0.0236
Ruminal Mean retention time (h)	29.33	28.32	0.009	0.0026
Milk yield (kg/day)	21.00 ^b	22.54 ^a	0.310	0.0014
Milk fat (kg/day)	0.75 ^b	0.81 ^a	0.012	0.0009
Milk protein (kg/day)	0.51 ^b	0.57 ^a	0.005	<0.0001

Conclusion Microbial inoculation of corn silage can improve the dry matter intake and performance of Holstein dairy cows.

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Effects of starch level and forage particle length on the performance, milk fatty acid profile and production of *trans*-10, *cis*-12 conjugated linoleic acid in dairy ewes

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Introduction Feeding high starch, low fibre diets to ruminants has been shown to decrease milk fat content (Choi *et al.* 2005). This effect may be due to the production of *trans*-10, *cis*-12 CLA in the rumen, which is a known inhibitor of milk fat synthesis in ruminants. Forage particle length may also have an influence on milk fat synthesis by altering ruminal pH (Beauchemin *et al.*, 2003) and subsequently the production of *trans*-10, *cis*-12 CLA. The objectives of the current study were to evaluate the effects of starch content and forage particle length on the performance and production of *trans*-10, *cis*-12 CLA in dairy ewes.

Materials and methods Twelve multiparous ewes and their lambs were grouped immediately post lambing and fed a standard ewe concentrate with grass hay offered *ad-libitum*. At 4 weeks post-partum the lambs were weaned, the ewes individually penned on sawdust and machine milked at 0800 and 1530 h. At the 5th week of lactation ewes were randomly allocated to one of four treatments based on their milk yield and composition in the week prior to allocation. Ewes were fed one of four complete diets (0.55 concentrates: 0.45 hay; DM basis) *ad-libitum*, that contained one of two starch levels (high; 168 g/kg DM or low; 53 g/kg DM) and forage particle length (long; 19 mm or short; 3 mm). Diets were therefore: high starch long forage (HL), high starch short forage (HS), low starch long forage (LL) and low starch short forage (LS). The diets were fed in a 4 X 4 Latin square design with a 20 d adaptation period followed by a 5 d sampling period, during which milk yield was recorded daily and samples taken for analysis of fat and protein. On the 5th day of each sampling period, an additional milk sample was taken for subsequent fatty acid analysis and on the 3rd day, rumen fluid pH was measured 3 times at 7.30 am, 11.30 am and 2.30 pm. Data were analysed as a 2 X 2 factorial design with main effects of starch and forage length and their interaction using Genstat 8 (VSN Int. LTD., Oxford, UK).

Results There was no effect ($P > 0.05$) of forage particle length on dry matter intake, but ewes fed the high starch diets had a greater ($P < 0.05$) DMI than those fed low starch. Milk yield, fat yield and protein content and yield were not affected ($P > 0.05$) by treatment. By contrast, milk fat content was proportionally 0.08 less ($P < 0.05$) in ewes fed the high compared with the low starch diets. There was no effect ($P > 0.05$) of treatment on milk fatty acid yield of > C16 or *trans*-10, *cis*-12 CLA. Milk fatty acid yield of < C16 was higher ($P < 0.001$) and C16 and C16:1 *cis*-9 lower in ewes fed the high compared to the low starch diets. Rumen fluid pH was lower ($P = 0.03$) in ewes fed the high compared to the low starch diets and tended to be higher ($P = 0.054$) in ewes fed the short compared to the long forage.

Table 1 Milk yield and composition of ewes fed diets containing high starch long forage (HL), high starch short forage (HS), low starch long forage (LL) and low starch short forage (LS).

Variable	Treatment				s.e.d	Significance ¹		
	HL	HS	LL	LS		S	F	S X F
Dry matter intake, kg/d	2.41	2.62	2.35	2.33	0.133	0.026	0.261	0.163
Milk yield, g/d	897	999	925	900	50.1	0.330	0.288	0.084
Milk fat:								
g/kg	62.0	60.5	67.0	65.6	2.90	0.021	0.474	0.988
g/d	55.8	60.2	61.0	58.0	2.95	0.492	0.723	0.089
Milk protein:								
g/kg	51.2	50.7	51.6	50.8	1.02	0.678	0.390	0.832
g/d	45.2	50.0	46.7	44.6	2.84	0.294	0.475	0.068
Milk fatty acids (g/100g)								
< C 16	23.1	25.1	20.8	20.5	0.936	<0.001	0.224	0.102
C16 and C16:1 <i>cis</i> -9	25.0	24.4	28.0	27.6	0.617	<0.001	0.258	0.874
> C 16	42.1	41.5	41.5	42.1	1.145	0.932	0.936	0.471
<i>Trans</i> -10, <i>cis</i> -12 CLA	0.03	<0.01	<0.01	0.01	0.017	0.470	0.413	0.146
Rumen fluid pH ²	6.93	7.09	7.08	7.19	0.174	0.031	0.054	0.747

¹S = Starch, F = Forage, S x F = Interaction between starch and forage

²Average rumen fluid pH, measured at 7.30 am, 11.30 am and 2.30 pm in the same day.

Conclusions Forage particle length had no effect on milk fat content or yield in dairy ewes. Feeding a high starch diet had a small, but significant effect on reducing milk fat content but neither milk fat yield nor the concentration of *trans*-10, *cis*-12 CLA were significantly affected. In dairy ewes, milk composition is therefore relatively unresponsive to dietary starch level or fibre particle length.

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The dose effect of rumen protected *trans*-10, *cis*-12 CLA on milk fat synthesis in dairy ewes

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Introduction *Trans*-10, *cis*-12 conjugated linoleic acid (CLA), a biohydrogenation intermediary produced in the rumen, has been shown to be a potent inhibitor of milk fat synthesis in dairy cows. Lock *et al.* (2006) demonstrated that milk fat synthesis in sheep was reduced to a similar extent as in dairy cows when a supplement containing CLA was fed at an equivalent level (metabolic body weight basis), although only one level of CLA inclusion was investigated. The objectives of this study were to examine the effects of level of inclusion of a ruminally protected source of *trans*-10, *cis*-12 CLA on milk fat synthesis and performance of dairy ewes.

Materials and methods Twenty four multiparous ewes that weighed 56 (\pm 8.8) kg were individually penned, the lambs weaned at 48 h post-partum and the ewes machine milked at 0800 and 1600h. At 10 d postpartum ewes were randomly allocated to one of three dietary treatments based on their performance prior to allocation. Ewes were fed a basal complete diet (0.55 concentrates:0.45 hay; DM basis, metabolisable energy content of 11.0 MJ/kg DM and crude protein content of 156 g/kg DM) *ad libitum* for 10 weeks, and were supplemented with a lipid-encapsulated CLA (BASF AG, Ludwigshafen, Germany) at one of three levels: no CLA (Control), low CLA (L-CLA) or high CLA (H-CLA). The lipid-encapsulated CLA supplement supplied approximately 1.4 or 3.8 g/d of *t*-10,*c*-12 CLA for L-CLA and H-CLA respectively. The supplement was weighed out for each ewe on a daily basis and was consumed within 5 min of being offered. Milk yield was recorded weekly and samples taken for analysis of fat and protein. Milk samples were also taken for fatty acid analysis during week 10 of the study. Data were analysed by ANOVA as a randomised block design using Genstat 10.1 (VSN Int. Ltd., UK).

Results There was no effect ($P > 0.05$) of treatment on DM intake, but ewes fed H-CLA had a 13% higher ($P < 0.05$) milk yield than those fed the Control or L-CLA. Milk fat content (g/kg) was reduced incrementally by CLA inclusion, being 15% lower in ewes fed L-CLA and 34% lower in those fed H-CLA compared with control animals ($P < 0.001$). Compared with the control, the reduction in milk fat yield (g/d) was 14% and 24% for L-CLA and H-CLA respectively. There was no effect of treatment on milk protein content but daily yield tended ($P = 0.07$) to be higher in ewes fed H-CLA compared with the control. The yield of all milk fatty acids was reduced incrementally with CLA supplementation, but the reduction was greatest for *de novo* synthesized fatty acids; as a consequence the profile of milk fat shifted to contain increased long-chain fatty acids. *Trans*-10, *cis*-12 CLA was undetectable in milk fat of unsupplemented animals (< 0.01 g/100 g fatty acids) and increased to 0.30 g/100 g fatty acids in animals fed H-CLA, with corresponding transfer efficiencies of CLA from the lipid-encapsulated supplement into milk of 2.5% (\pm 1.11) for animals fed L-CLA and 3.4% (\pm 1.12) for those fed H-CLA.

Table 1 Milk yield and composition of ewes fed a basal diet (Control) that was supplemented with a rumen protected source supplying either 1.4 g/d (L-CLA) or 3.8 g/d (H-CLA) of *trans*-10, *cis*-12 conjugated linoleic acid.

	Control	L-CLA	H-CLA	s.e.m	P-value
DM intake, kg/d	2.19	2.14	2.15	0.075	0.856
Milk yield, g/d	1268	1241	1433	52.6	0.046
Milk fat					
g/kg	73.7	63.0	49.0	1.82	<0.001
g/d	92.5	79.2	70.6	4.35	0.011
Milk protein					
g/kg	51.0	50.9	51.0	0.68	0.993
g/d	64.0	62.7	72.4	3.01	0.072
¹ Fatty acids (g/100g)					
<C16	27.1	23.1	17.8	1.61	<0.001
C16 and C16:1	32.9	32.8	30.7	0.99	0.065
>C16	34.1	37.9	44.7	1.69	<0.001
<i>t</i> -10, <i>c</i> -12 CLA	0.00	0.07	0.30	0.021	<0.001

¹Measured during week 10 of the study

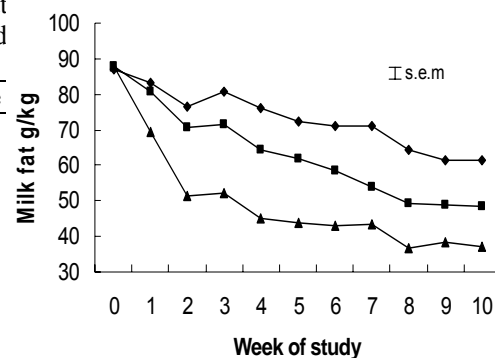


Figure 1 Weekly milk fat content of ewes unsupplemented (□) or supplemented with a rumen protected source supplying 1.4 g/d (○) or 3.8 g/d (▲) of *t*-10,*c*-12 CLA. Values represent means from 8 ewes per treatment.

Conclusions The results of this study show a dose dependent reduction in milk fat content and increase in *trans*-10, *cis*-12 CLA transfer from the rumen protected supplement into milk. At the higher level of supplementation, the energy spared by the reduction in milk fat coincided with a large increase in milk yield. Further studies are required to determine the partitioning of the spared energy from CLA-induced milk fat depression and to elucidate the nature of regulation through which these effects occur.

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Effect of season of shearing on ewe and progeny performance

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Introduction Ewes are normally shorn once yearly, usually in early summer, to maintain sheep welfare and to minimize fly strike. Winter conditions in Ireland are characterized as relatively mild. Consequently ewes which are housed unshorn may have difficulty dissipating body heat due to the unique insulating properties of the fleece, leading to ineffective heat regulation. Results from previous studies at Athenry (Keady *et al* 2007, Keady and Hanrahan 2007) showed that shearing at housing increased lamb birth and weaning weights by up to 0.63 and 2.5 kg respectively. Shearing at housing may require a greater management input as ewes are normally housed in smaller groups and need to be dry prior to shearing. However, shearing in the autumn prior to mating enables the total flock to be assembled under more favourable conditions. The number of lambs weaned per ewe mated is the major factor influencing efficiency of prime lamb production (Keady and Hanrahan 2006). It is unknown if shearing prior to mating, in a temperate climate, impacts on subsequent ewe fertility and litter size whilst at the same time producing heavier lambs relative to shearing at the conventional time in early summer. The aim of this study was to evaluate the effect of the season of shearing on ewe fertility of March lambing ewes and subsequent lamb birth and weaning weights.

Materials and methods One hundred and thirty ewes [(Belclare x Scottish Blackface, Chamoise x Scottish Blackface)(66 first crop, 64 second crop)] were allocated to four shearing treatments as follows: Conventional (C), prior to mating (M), housing (H) and twice yearly (MH) and were shorn on 29 May, 9 September, 30 November and 29 May and 9 September, respectively. The ewes on the M and H treatments had been shorn the previous December whilst the ewes on the C and MH treatments had been shorn the previous May. The ewes were managed as one flock from the parturition prior to the study. All ewes had a synchronised oestrus (using progesterone impregnated sponges) prior to joining the rams on 9 October for syndicate mating. The ewes were housed in slatted pens during the winter feeding period and offered silage based diets supplemented with a total of 21 kg concentrate during the last 6 weeks prior to lambing. The ewes were turned out to pasture within 3 days of lambing. Triplet rearing ewes received 1 kg concentrate daily for 5 weeks post lambing and triplet lambs were offered concentrates to a maximum of 300 g/day from birth to weaning. Ewes rearing single or twins and their lambs received no concentrate post lambing. Lambs were weaned at 14 weeks of age. Animal performance data were analysed using Proc GLM and Proc MIXED of SAS as appropriate.

Results The effects of shearing treatment on ewe and subsequent lamb performance are presented in Table 1. Ewes shorn twice yearly had a significantly higher condition score pre-mating than ewes on the other three treatments. Treatment did not alter ($P > 0.05$) condition score at lambing, litter size or number of lambs reared per ewe joined. Lambs from ewes shorn at housing were heavier ($P < 0.001$) at birth relative to lambs from ewes shorn at the conventional time or twice yearly. Lambs from ewes shorn prior to mating and at housing tended to be heavier at weaning ($P=0.09$) relative to lambs from ewes shorn at the conventional time.

Table 1 The effects of shearing treatment on ewe and subsequent lamb performance

	Shearing treatment				s.e	sig
	Conventional	Prior to mating	Housing	Twice yearly		
Ewe condition score at:						
pre-mating	3.40 ^{ab}	3.49 ^b	3.34 ^a	3.60 ^c	0.037	***
lambing	4.02	3.99	3.73	3.96	0.130	NS
Litter size	2.02	2.17	2.04	2.05	0.107	NS
Lambs reared per ewe joined	1.71	1.97	1.94	1.83	0.132	NS
Birth weight (kg)	4.27 ^a	4.58 ^{ab}	4.81 ^b	4.43 ^a	0.102	***
Weaning weight (kg)	30.7	32.7	32.0	31.4	0.62	P=0.09

Conclusions Shearing prior to mating provides an alternative to shearing at housing.

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The effects of grass silage harvest system, concentrate feed level and maize silage maturity and soyabean supplementation on ewe and subsequent lamb performance

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Introduction Ewes housed during the winter feeding period are predominantly offered grass silage as the sole forage. Approximately 55% of grass silage is ensiled in big bales on Irish sheep farms. Maize silage can be produced at a similar cost to grazed grass and approximately 20% cheaper than grass silage (Keady *et al.* 2002a). Recent studies have shown that maize silage inclusion in the diet increases the performance of dairy (Keady *et al.* 2002b, 2003) and beef (Keady and Gordon 2006, Keady *et al.* 2007) cattle. The objective of the present study was to evaluate the effects of grass silage harvest system, maturity of maize at harvest and protein supplementation of maize silage diets on the performance of ewes in mid and late pregnancy and subsequent lamb performance. The potential concentrate sparing effect of maize silage was also determined.

Materials and methods Four grass silages were harvested from the primary growth (H1) and regrowth (H2) of predominantly perennial ryegrass swards and ensiled either precision chopped (PC) or in big bales (BB). Two maize silages were produced, either grown in the open [sown on May 10 (LDM)], or under the complete plastic mulch system [sown on April 10 (HDM)] and harvested on the same date. Each grass silage was offered *ad libitum* and was supplemented with either 18 or 27 kg concentrate in late pregnancy whilst the maize silages were offered *ad libitum* and supplemented with either with 0 or 200 g soyabean meal/ewe daily and 18 kg concentrate in late pregnancy. The 12 treatments were offered to 180 ewes (Belclare x Scottish Blackface, Chamoise x Scottish Blackface), during mid and late pregnancy, penned in groups of five, using 3 replicates per treatment. The data was analysed using Proc GLM and Proc MIXED of SAS.

Results The chemical composition of the grass and maize silages are presented in Table 1. The effects of grass silage harvest system, maturity of maize at harvest and soyabean supplementation on ewe and subsequent lamb performance are summarised in Table 2. Relative to ensiling in big bales, precision chopping increased subsequent lamb growth rate, weaning weight and number of lambs reared. Increasing concentrate feed level in late pregnancy increased lamb birth weight. Increasing maturity of maize at harvest tended to decrease lamb birth weight ($P=0.08$). Ewes offered precision chopped silage tended ($P=0.06$) to rear larger litters relative to ewes offered high dry matter maize silage. There were no effects ($P>0.05$) of forage type or soyabean supplementation of maize silage on ewe or subsequent lamb performance. The potential concentrate sparing effect of LDM and HDM maize silages, as determined by lamb weaning weight were approximately 3 and 16.5 kg and -11 and -2.5 kg relative to baled and precision chopped silages, respectively.

Table 1 Chemical composition of the grass and maize silages

	Grass silage				Maize silage	
	H1 BB	H1 PC	H2 BB	H2 PC	LDM	HDM
Dry matter (g/kg)	236	221	273	265	247	392
Predicted ME (mj/kg DM)	11.3	11.1	11.8	11.7	11.0	11.5
Starch (g/kg DM)	-	-	-	-	200	270

Table 2 The effects of treatment on ewe and subsequent lamb performance

	Litter size	Number reared	Birth weight(kg)	Weaning weight (kg)	Gain(Birth-Weaning)(g/d)
Mean	2.0	1.93	4.74	31.9	278
Conc. feed level: 18v27 kg	+0.20±0.115	-0.04±0.089	+0.31±0.127*	0.90±0.672	+7.9±6.30
Harvest system: bale v precision	+0.05±0.114	+0.18±0.088*	+0.06±0.124	1.80±0.672**	+16.9±6.31**
Maize type: LDM v HDM	-0.25±0.159	-0.19±0.124	-0.31±0.176	+1.06±0.972	+12.2±9.12
Soya with maize: no v yes	+0.05±0.159	+0.029±0.123	+0.23±0.178	-0.18±0.984	-2.9±9.23
Forage type: maize v grass	+0.20±0.143	+0.10±0.111	+0.17±0.160	-0.05±0.87	-2.7±8.15
Harvest number:	+0.18±0.114	+0.00±0.088	+0.06±0.124	+0.40±0.679	+4.4±6.36

Conclusions Regardless of stage of maturity at harvest, maize silage resulted in the same level of subsequent lamb performance as high feed value grass silage. Ensiling grass in big bales reduced subsequent lamb performance relative to precision chopping. The potential concentrate sparing effect of maize silage was up to 16.5 kg/ewe, depending on maturity at harvest and grass ensiling system.

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Plasma concentrations of glucagon-like peptide-1 and cholecystikinin in sheep are altered by the amount of dry matter intake and the addition of fat to the diet

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Introduction Dry matter intake (DMI) regulation is important in ruminants as DMI is a major determinant of milk and meat production. It is known that the gut peptides glucagon-like peptide-1_{7,36} amide (GLP-1) and cholecystikinin-8 (CCK) can reduce DMI in nonruminants (Walsh, 1994). In ruminants, there is little information on the function or effects of diet on plasma concentrations of these peptides. Previous studies have shown that supplemental dietary fat (Relling and Reynolds, 2007b) increases plasma concentrations of GLP-1 and CCK and decreases DMI. In contrast, increased metabolizable energy intake (MEI) in early lactation also increases GLP-1 and CCK concentration in dairy cows (Relling and Reynolds, 2007a). Therefore, our objective was to determine if plasma concentrations of GLP-1 and CCK are affected by supplemental fat, and if the response differs for *ad libitum* versus restricted DMI.

Materials and methods Sixty four Targhee x Hampshire wethers were blocked by body weight (BW) and used in a completely randomized block design study with 2 consecutive 29-day periods (32 wethers per period). Within each period lambs were housed in 16 pens with 2 lambs per pen blocked by BW (8 heavy and 8 light BW pens). A pelleted ration (400 g/kg forage, 600 g/kg concentrate per kg dry matter [DM]) was fed daily at 0800 h. Daily DMI was measured and MEI estimated based on diet composition. The experimental design had a 2 x 2 factorial arrangement of treatments with feeding (intake effect: restricted versus *ad libitum*) and supplemental fat (fat effect: control versus 60 g/kg of ration DM as Ca salts of palm oil) as main effects. Restricted wethers assigned to the control diet were fed a fixed amount of diet DM equal to 25 g/kg of average pen BW at the start of the study. Restricted wethers fed supplemental fat were fed for equal estimated MEI to the restricted wethers on the control diet (22.65 g/kg of BW). Wethers fed for *ad libitum* DMI were fed either the control or fat supplemented diet for 10% refusals. The control diet was fed to all lambs for *ad libitum* DMI for 15 days prior to treatments. Supplemental fat was added incrementally over the first 4 days. Refusals were collected daily at 0700 h and BW was measured on day 1, 8, 15, 22 and 29 at 0730 h. Weekly feed samples were pooled for each period and analyzed for DM (100°C). Blood samples from the jugular vein were collected 6 h after feeding on day 8, 15, 22 and 29 and processed, stored and analyzed for GLP-1 and CCK as described previously (Relling and Reynolds, 2007a). Pen averages (the experimental unit) of dependent variables were statistically analyzed as repeated measures using mixed models procedures testing the random effect of pen and fixed effects of block, fat, intake, and fat by intake interaction. The restricted treatments were not included in the analysis for DMI and MEI as these were dictated by the experimental design and were not independent variables.

Results Restricting DMI (and therefore MEI) reduced BW (Table 1; $P < 0.01$), due to a decrease in growth rate, independent of the presence of fat in the diet ($P > 0.05$). The inclusion of fat in the diet numerically decreased DMI and MEI when wethers were fed for *ad libitum* DMI, but the decreases were not significant. Plasma GLP-1 concentration was increased by higher intake level ($P < 0.01$) and by feeding supplemental fat ($P < 0.01$). A fat by intake interaction was observed for plasma CCK concentration ($P < 0.05$). Supplemental fat resulted in a greater increase in plasma CCK concentration when lambs were fed for *ad libitum* DMI than when intake was restricted.

Table 1 Effects of intake level and supplemental fat on DMI (kg/d), MEI (MJ/d), BW (kg), and plasma concentrations (pM) of GLP-1 and CCK in wethers

Item	Restricted		<i>Ad libitum</i>		s.e.m.	$P <$		
	Control	+ Fat	Control	+ Fat		Fat	Intake	Fat x intake
BW	37.0	36.7	42.8	42.5	0.40	0.480	0.001	0.951
DMI	0.94	0.85	1.97	1.74	0.08	0.124	-	-
MEI	10.29	10.29	21.76	20.96	0.96	0.198	-	-
GLP-1	21.2	24.8	29.2	38.5	1.8	0.002	0.001	0.145
CCK	4.95	6.08	9.15	15.55	1.94	0.006	0.001	0.042

Conclusions Plasma GLP-1 concentration increased due both to increased feed intake and the addition of fat to the diet. Plasma CCK concentration also increased when wethers were fed for *ad libitum* intake, but the effect of adding fat to the diet on CCK concentration was greater at *ad libitum* intake. From these data it is clear that plasma concentrations of GLP-1 and CCK respond positively to both increases in DMI and supplemented fat, but their role in the regulation of DMI is not certain.

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Effect of raw or roasted whole soybean on early lactational performance of Iranian Holstein cows

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Introduction Whole soybeans (SB) are mostly used in feeding of dairy cows, but protein in raw SB is degraded readily by rumen microbes and cannot meet the high demand of rumen undegradable protein (RUP) in early lactation. Heat treatment of SB, especially roasting, reduces ruminal protein degradation and improves the nutrient balance for cows. However, there have been conflicting results regarding animal response to feeding heat treated SB. In many studies, increase in milk yield may be related to type of forage in the diet, as improved performance is usually achieved by diets with lucerne silage as the forage because of the high solubility of its protein, but mixed lucerne hay-maize silage as the primary forage source of diets that are used widely in tropical areas like Iran, was not tested. So, the main objective of this study was to investigate the performance responses of Iranian Holstein cows in early lactating to the feeding of Iranian roasted SB, raw SB and SBM in combination with lucerne hay-maize silage diets.

Materials and methods Fourteen multiparous Holstein cows were housed after calving in tie stall barns for 2 weeks for barn adjustment and collection of pre-trial data. At the onset of the 45-d trial, cows were grouped according to pre-trial milk yield and then randomly assigned from these subgroups to one of three experimental diets. Cows in each group were fed individually one of three experimental diets as totally mixed ration. The diets, which contained 120g/kg soybean meal (SBM) plus 82g/kg cottonseed (CS), 120g/kg raw SB plus 82g/kg cottonseed meal (CSM) and 120g/kg roasted SB plus 82g/kg CSM were formulated to be iso-nitrogenous and iso-caloric and meet NRC (NRC 2001) recommendations. Roasted SB were obtained by roasting seeds in a commercial roaster (exit temperature of seeds was about 140 - 145°C) and immediately placing, in covered wooden barrels for 45 min. Milk yield was recorded daily and samples were collected at each milking weekly. Milk samples were combined on an individual cow basis and analyzed for fat, protein, lactose by infra-red analyzer. Data were analyzed by ANOVA and repeated measures using the MIXED procedure of SAS.

Results Dry matter intake was significantly higher for cows fed roasted SB diet than for cows fed raw SB diet but there was no significant difference between SB plus CSM diets with SBM plus CS diet. Cows fed roasted SB consumed significantly higher RUP than the cows fed raw SB. Cows fed roasted SB produced 1.3 kg/d more milk throughout the study than cows fed raw SB and cows fed SB plus CSM diets produced 1.4 kg/d more milk than cows fed SBM plus CS diet. This corresponds to an increase in yield of 3.5% FCM to 1.2 and 1.3 kg/d, respectively. Milk fat concentration was similar among diets but milk fat yield was higher for cows fed roasted SB compared with cows fed raw SB (40.0 g/d) and cows fed SB plus CSM compared with SBM plus CS (40.0 g/d). Milk protein yield was similar for cows fed different diets. Milk protein concentration was the same for cows fed raw SB diet and roasted SB diet, but depressed by 1.8 g/kg for cows fed SB plus CSM diets compared with SBM plus CS diet (Table 1).

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

Item	SBM + CS	Raw SB + CSM	Roasted SB + CSM	SEM	Contrast ¹	
					1	2
<i>P</i> values						
Intake						
DM, kg/d	22.3	22.0	23.3	0.29	0.01	NS ²
DM, % of BW	3.64	3.62	3.77	0.048	0.04	NS
RUP (calculated), kg/d	1.43	1.39	1.68	0.021	0.01	0.01
Yield, kg/d						
Milk	37.9	38.6	39.9	0.38	0.03	0.02
3.5% FCM	35.7	36.4	37.6	0.34	0.02	0.01
Fat	1.19	1.21	1.25	0.010	0.02	0.01
Protein	1.14	1.10	1.12	0.010	NS	NS
Fat, g/kg	31.6	31.2	31.4	1.34	NS	NS
Protein, g/kg	30.1	28.5	28.1	0.36	NS	0.01
Production efficiency ³	1.60	1.67	1.62	0.019	NS	NS

¹ Contrast includes 1) roasted SB vs. raw SB and 2) SB plus CSM vs. SBM plus CS; ² $P > 0.05$; ³ Production efficiency = average daily 3.5% FCM (kg/d)/ average daily DMI (kg/d).

Conclusion Feeding roasted SB treated to maximize RUP supply to the intestine supported higher milk and FCM production in early lactation cows fed lucerne hay and maize silage as the primary forage source of diet. Furthermore, the higher milk and 3.5% FCM production of cows fed SB plus CSM diets compared with cows fed SBM plus CS showed the CSM can act as a suitable protein source in SB included diets. Although it was reduced in some studies where dietary forage comprised solely or partly maize silage, there was no significant difference between diets in milk fat concentration in this experiment, and heat treatment applied to SB provided additional benefits over raw SB. Because of the inclusion of cottonseed (meal or whole seed) in all diets, it did not confound the SB diets.

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Effect of diets containing different levels of steam treated pith bagasse on performance and carcass characteristics of fattening Arabi lambs

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Introduction Pith bagasse one of industrial agriculture's by product that is produced by several sugar cane factories in south west of Iran. The low dry matter digestibility of pith bagasse is influence by crud fibre content and is the reason of the poor quality. It is claimed that low dry matter digestibility improve by steam pressure hydrolysis (Medeiros *et al.* 1993, Pessoa *et al.* 1997). There isn't adequate information about the effect of hydrolyzed pith bagasse in ruminant nutrition. Therefore the aim of this experiment was to examine the effect of dietary containing different levels of pith bagasse on performance and carcass characteristics of fattening Arabi lambs.

Materials and methods Forty male lambs with similar condition were used in completely randomized design. The lambs were divided, at random in four groups (10 lambs in each pen). Four diets were formulated. The diet 1 (control group, 0% pith bagasse) formulated according NRC for 300 g daily gain as a standard. The total hydrolyzed pith bagasse content of diets 1-4 were respectively 0, 11, 22 and 33%. The diets were offered ad libitum to all groups. Lamb live weights were measure from beginning and 2 week interval for a period of 90 days. At the end of experiment 3 lambs from each treatment were slaughtered and carcass characteristics were measured. The right half was divided into the cuts: neck + proximal thoracic limb + steaks + brisket, lumbar + abdominal region, proximal pelvic limb, and weights of each cut were recorded. The weights of liver, kidney, visceral fat and carcass meat, bone and fat tail percentage were measured separately. Data were analyzed as a completely random design using the GLM procedures of SAS[®] (SAS Institute, 1999) 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 The results of this experiment are shown in Table 1. Total average daily gain (ADG) (307 vs. 267.7, 251 and 226) of fattening lambs fed the diets containing 11% pith bagasse was significantly higher ($p < 0.05$) than lambs fed the control diet and diets containing 22 and 33% pith bagasse. Diet 4 (containing 33% pith) had significantly lower ADG than control group ($p < 0.05$). Feed conversion ratio (FCR) of lambs fed diet 2 (containing 11% pith bagasse) better than others. Carcass percentage (CP) in the diet 2 was the highest. The main carcass characteristics were not affected by dietary treatment.

Table 1 Dry matter intake (DMI), ADG, FCR and CP of lambs during whole period of experiment

	1(0%pith bagasse)	2(11%pith bagasse)	3(22%pith bagasse)	4(33%pith bagasse)
DMI* (g/day)	1400	1441	1389	1466
ADG(g/day)	267.7 ^{ab} ± 19	307 ^a ± 19	251 ^b ± 42	226.1 ^c ± 28
FCR(g DMI/g daily gain)	5.24 ^b ± 0.2	4.7 ^b ± 0.2	5.5 ^{ab} ± 0.7	6.5 ^a ± 0.6
CP (%)	43.36 ^{ab} ± 3.6	48.2 ^a ± 1.38	46 ^{ab} ± 2.5	41.9 ^b ± 1.3

a,b,c means in row with different superscripts differ significantly ($p < 0.05$)

*Because of feed were not offered to all groups individually, so the cases to depend feed intake in periods were not analyzed.

Conclusions Despite non significant differences between ADG of control group with lambs fed diet 2 and 3, but lambs fed diet 4 had significantly ($P < 0.05$) lower ADG than control group. So lambs fed diet 2 (containing 11% hydrolyzed pith bagasse) had the best performance. Moreover until 22% pith bagasse (diet3) can be used without any negative effects, but utilization over than this level (diet 4) lead to decrease in FCR and ADG of lambs. This study suggests that lambs fed diet 3 had also better net return than control group which is important for Iran condition. So it concluded that pith a byproduct of sugarcane has potential as an alternative feed source for ruminants, particularly for fattening lambs.

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Computerised machine rearing systems for group housed dairy-bred bull calves

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Introduction There are numerous calf rearing systems but the last decade has seen increased interest in the use of computerised machine feeders for group reared calves in place of systems based on bucket rearing with individual housing. Automated machines are expensive but are becoming more common due to reductions in labour and advances in technology allowing regulation of milk intakes (Hepola, 2003). The objective of this experiment was to evaluate the effect of rearing dairy-bred bull calves on either a computerised machine or twice a day milk feeding system to weaning at 6 weeks old.

Materials and methods Thirty six Holstein-Friesian bull calves were assigned in a randomised block designed experiment to weaning on either a twice a day milk feeding system or computerised machine (DeLaval CF150). The calves started the trial at 1 day of age and were individually penned on straw. From days 1 to 4 they were fed colostrum. The twice a day milk fed calves (Bucket) were individually housed and fed a whey based milk replacer (DM 938g/kg, 218g crude protein/kg, 181g/kg Ether Extract [Wynngold Calf Milk Replacer, Wynnstay Group plc]) mixed at 125g per litre of water at 40°C twice per day in buckets. From 5 to 7 days they were fed 4 litres per day and from day 8 to 28 milk was fed at 5 litres per day. Thereafter the milk feeding rate was gradually reduced to weaning at 42 days old. The computerised machine fed calves (Machine) were group housed and fed the same milk powder at an identical daily feed rate available throughout the day with a maximum intake of 60% of milk available in the first feed with the remainder available 2 hours later. From day 4 the calves on both treatments received *ad libitum* concentrates (Start 'n' Wean pellets, Wynnstay Group plc) plus straw and water. The behaviour of the calves was recorded by video over a series of 24 hour periods. The individually reared bucket fed calves were thus unable to cross suckle. The calves were moved into group pens at weaning. The data was analysed by ANOVA with calves blocked according to weight.

Results There were no significant differences in live weight, DLWG (see table 1), feed intakes or health of the calves.

Table 1 Effect of milk feeding system on liveweight (kg)

	Bucket	Machine	s.e.d	Sig
Start weight	45.2	47.3	2.15	NS
3 week weight	53.5	55.1	1.83	NS
Weaning weight	61.6	62.7	2.35	NS
11 week weight	102.1	102.2	4.06	NS

Table 2 Effect of milk feeding system on behaviour

Characteristic	Bucket	Machine	s.e.d	Sig
Cross sucking - mouth	0	1.67	0.0007	*
Cross sucking - navel	0	0.33	0.374	NS
Cross sucking – ear and other	0	2.67	0.016	*
Looking	10	4.33	0.027	*
Tactile contact	1.3	3.7	0.336	NS
Play	1.3	3.7	0.011	*
Idle standing	27	14	0.055	=0.072
Lying down	95	110	0.803	NS
Restlessness	23.3	11	0.004	*
Grooming	4	2.33	0.346	NS
Licking	3.33	2.33	0.417	NS
Feeding	14.3	27	0.006	*

The machine fed calves recorded significantly higher ($p < 0.05$) incidence of mouth, ear and other body part sucking, play and feeding. The individually reared bucket fed calves recorded significantly higher ($p < 0.05$) incidence of looking and restlessness compared to the group reared machine fed calves. The time spent attending each calf for feeding, bedding and checking was 152 and 71 minutes from start to weaning for the bucket and machine systems respectively. If labour is valued at £10 per hour this costs £25.34 and £11.90 per calf, a reduction of £13.44 in rearing costs for the machine fed calves.

Conclusions There were significant differences in behaviour between the two treatments with the machine reared calves recording significantly higher incidence of body part sucking, play and feeding. The bucket reared calves recorded significantly higher incidence of looking and restlessness compared to the machine reared calves. Does the latter indicate that the machine reared calves were 'more content'? Labour inputs were reduced by 53% with the machine reared calves. The DeLaval CF150 feeder costs approximately £3,000 and this would be recouped by rearing over 223 calves.

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Effect of forage type and enzyme feed additive on performance of Iranian Holstein cows

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Introduction Enzymes are used to improve the nutritive value of feeds for nonruminants. Many experiments showed that enzymes substantially improved feed digestibility and animal performance in sheep and cattle, but results were often inconsistent (yang *et al*, 1999). Various results from recent studies can be contributed to a number of factors including diet composition, type of enzyme preparation, complement of enzyme activities, amount of enzyme provided, enzyme stability, and method of application (Schingoethe *et al*, 1999). Some experiments showed that type of forage and grains in rations can be as an important factor for effecting enzyme in diets (Beauchemin *et al*, 1999). The objectives of this experiment were to determine the effects of type of forage (Corn silage vs. barley silage) in diets with and without mixture enzyme in the diets of dairy cows on food intake, milk production, and milk production.

Materials and methods Eight lactating Holstein cows were used in this study. At the start of the experiment, cows averaged 68.4±5.8 DIM and 43.8±0.6 milk production were housed in individual tie stalls. Cows were milked three times daily 05.00, 13.00 and 21.00. Cows were offered total mixed rations two times daily at 08.00 and 17.00 for ad libitum intake. They were turned out side 3 hours daily except during digestibility measurements. The experimental design was replicated 4×4 Latin square with four 21 days periods. Each period consisted of 14 days adaptation to diets and 7 days of experimental measurements. Each period, cows received one of four diets. The four diets were consisted of 35% forages include 20% alfalfa hay and 15% barley silage or corn silage. The concentrate contain 1 gr of enzyme per 1 kg DM. the enzyme mixture was a mixture of cellulase, xylanase, B-glucanase, A-amylase, protease, pectinase and phytase (NATUZYME, Bioproton, Au). Feed offered andorts were measured and recorded daily to calculate feed intake. Milk samples were taken two last days of each experimental period and sent to the central Mashhad milk testing laboratory for milk fat, cp and lactose determination. Data were analyzed using the GLM procedure of SAS 9.1 to determine the effects of cow, period and diet. Means were compared using Duncan test (P<0.05).

Results Table 1 show DMI, Milk yield and milk fat, protein, lactose, and solid non fat percentage in cows offered different types of silage as forages with or without enzyme. DMI, protein, lactose, and solid non fat percentage showed no significant difference except fat percentage which was significantly higher for barley silage vs. corn silage. There were also no clear trends between treatments.

Table 1 Effect of forage type and exotic enzyme on DMI and milk constituents

Items	Treatments				SEM	A ¹	S ²	A×S
	Corn silage		Barley silage					
	No Enzyme	Enzyme	No Enzyme	Enzyme				
Dry matter intake (Kg/day)	23.64	23.71	23.57	23.63	6.28	0.71	0.62	0.58
Milk yield (Kg/day)	37.00	36.60	36.90	36.80	0.41	0.64	0.45	0.53
Milk fat (%)	3.18	2.98	3.36	3.38	0.14	0.62	0.05	0.52
Milk protein (%)	3.05	3.03	3.04	3.04	0.10	0.38	0.70	0.54
Milk lactose (%)	4.61	4.66	4.62	4.65	0.09	0.58	0.77	0.80
Solid non fat (%)	8.37	8.41	8.36	8.39	0.21	0.56	0.77	0.80

Items with different letter in each row have significant difference (P<0.05).

1- A: Additive pvalue 2- S: Silage pvalue

Conclusion It seems that barley silage had more effect on the milk fat percentage in comparison with enzyme supplementation.

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Effect of fat supplement in Holstein dairy cow transition diets on plasma leptin concentration and performances

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Introduction The effect of increasing energy supply through fat supplementation (Douglas *et al.*, 2004) in transition period on plasma leptin concentrations was not elaborated. The main objective of this study was to determine the effects of supplemental fat in transition cow diets, with differing caloric densities, on plasma leptin, glucose and NEFA concentrations.

Materials and methods Fifteen Holstein multiparous dairy cows were used. The close up diets consisted of 1) a low energy diet with no fat supplement (LD), 2) a moderate energy diet with 2% supplemental fat (MD), and 3) a high energy diet with 4% supplemental fat (HD). The diets were based on alfalfa and corn silage. The fat source was a hydrogenated rumen inert fat. The cows received a single lactation diet after parturition (30 days in milk) with 2% supplemental fat and 1.74 Mcal/kg NEL (net energy for lactation). The body weight (BW) and body condition score (BCS) were measured weekly during pre and postpartum. Prepartum dry matter intake and net energy intake, was recorded daily. Weekly estimated energy balance (EB), glucose, non-esterified fatty acids (NEFA) and plasma leptin concentrations, were determined pre and postpartum. Data were subjected to ANOVA for a randomized design with repeated measures using the MIXED procedure of SAS (Littell *et al* 1996; Release 9.0, SAS Institute Inc Cary NC).

Results The prepartum DMI (dry matter intake) was not affected by prepartum diets and also there were no significant differences in NEI (net energy intake) prepartum. Cows received MD and HD diets, had better energy status, and were in a more positive EB prepartum compared to those received LD diets ($P < 0.001$). After parturition, BW of cows fed HD diet was greater than LD and MD diets ($P < 0.05$). Postpartum BW and BCS tended to be greater for HD cows compare to LD diet ($P = 0.05$). No significant differences were found between treatments in plasma NEFA concentration, pre and postpartum ($P > 0.05$). Prepartum diets had significant effect on postpartum plasma glucose concentration ($P < 0.05$). After parturition, cows fed HD and MD diets prepartum, had significantly greater plasma glucose concentrations ($P < 0.05$). Prepartum plasma leptin concentration was significantly different across treatments ($P < 0.05$). In agreement with other studies (Kokkonen *et al* 2005), leptin concentration decreased as parturition approached ($P < 0.05$; Figure 1). There were no significant differences between plasma leptin concentrations postpartum.

Conclusions The results showed increasing energy density through fat supplementation near the time of calving could result in better energy balance pre and postpartum and it could affect prepartum leptin concentration and postpartum BCS and BW maintenance and lead to more milk production and lower metabolic disorders.

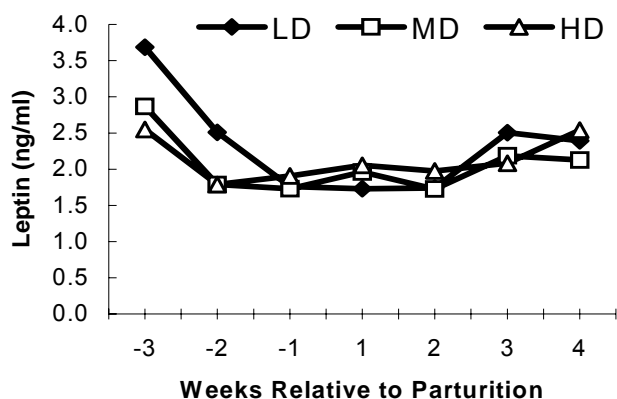


Figure 1 Plasma leptin concentration in cows receiving a low energy diet (LD, \blacklozenge); moderate energy diet (MD, \square) and high-energy diet (HD, \triangle) prior to calving.

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Initial investigations into feeding a combined vitamin and organic mineral supplement on the fertility of UK sport horse stallions

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Introduction Previous investigations have quantified the significant variability in frozen semen quality from sport horse stallions based primarily in the UK (Tucker *et al.*, 2007). Many competition stallions do not produce semen suitable for freezing, which limits the global distribution of their genetics. In this study, the second part of a series of trials, the influence of dietary supplementation with anti-oxidants on semen quality parameters was evaluated.

Materials and methods A pilot study was performed using six stallions selected from collections made at Stallion AI Services, UK between October 2006 and October 2007. Stallions were selected for the trial that did not have semen suitable for freezing. The horses received the same housing, management and premium commercial feed, with intake adjusted for body weight (which was monitored twice weekly throughout the collection period). 'Flushing' collections were made prior to the main collecting period, to remove senescent sperm. Semen was collected in an artificial vagina. Each batch was filtered to remove the gel fraction. Fresh semen volume and sperm concentration were measured, and progressive motility were determined by microscopy. Two collection periods per stallion were conducted in the 12 month period. During the second collection, the supplement was fed in addition to the standard feed, with a daily intake of 30 g of supplement (based on 500 kg bodyweight and adjusted accordingly) per horse. The data from the two collection periods was then compared. Each semen collection was treated as an individual result, and data was analysed by one-way ANOVA using the GLM process of UNISTAT, with confidence limits set at 5%.

Results Table 1 shows the outcome of feeding the supplement on the semen collections and sperm motility assessments. There were no significant effects of supplementation with anti-oxidants on the ejaculate volume, sperm concentration, or sperm motility parameters. A significant (13%) increase in the amount of extender required (used for standardised dilution, which is calculated based on sperm density and semen volume) was observed when the sires received the supplement ($P < 0.05$).

Table 1 Effect of a vitamin and organic mineral supplement on semen parameters from performance stallions

Parameter	Control	Supplement	P value	SEM
Semen volume (ml)	16.4	16.0	0.659	0.39
Sperm concentration ($\times 10^6$ /ml)	191.0	203.4	0.270	5.58
Extender required (ml)	37.8 ^a	42.8 ^b	0.0006	0.75
Motility (%)	66.2	66.2	0.932	0.31
Progressive motility (%)	58.4	60.0	0.135	0.525
No. progressive sperm ($\times 10^6$)	1776	1881	0.402	62.2

^{a, b} means not sharing a letter differ significantly ($P < 0.05$)

Conclusions A significant ($P < 0.05$) increase in extender required may illustrate the combined effects of the vitamin and organic mineral supplement on the overall production of viable sperm in horse semen. The results suggest that the combination of the numerically higher sperm concentration and progressive motility led more viable sperm per sample, resulting in the significantly higher dilution with extender (which is conducted to standardise the number of progressively motile sperm per ml). Further work is continuing regarding the post-thaw motility of these semen samples is required to demonstrate whether the supplement affected the robustness of the sperm. Following on from this work, the next trial will use Standardbred (trotting) stallions from New Zealand in a fully replicated trial, where the supplement is fed to the treatment group for one spermatogenesis cycle prior to collection.

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Voluntary feed intake, dry matter intake and consumption rate of three compositionally differing fibre sources when fed to veteran horses with compromised dentition

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Introduction In recent years, the importance of fibre provision within the horse's diet has been realised (Ralston 2005). Consideration of the evolutionary design of the animal, combined with a greater understanding of the aetiology of metabolic disorders, has re-iterated the need for horses to be fed diets high in fibre, especially those horses on maintenance rations. It has been acknowledged that although older horses can survive on maintenance rations, they are often at risk of weight loss (Ralston, 2005), a factor that is increased if dentition is poor (Dacre, 2005). Therefore nutritional management is vital to optimise nutrient provision. In dentally compromised animals, diet type and feed availability should be managed to promote ease of consumption, and subsequent factors compromising such consumption should be eliminated. Various methods of enhancing voluntary feed intake (VFI) have been devised (Dacre, 2005). Soaking hay may alleviate some problems associated with the ingestion and mastication of a course forage product, but can lead to consequences with decreased nutrient intake. Provision of softer fibre sources (haylage, silage) could also be considered, but will, conversely, increase nutrient intake. Short chopped fibre may decrease the overall need to chew. Other products, such as fibre pellets, may be fed soaked to horses with the most serious dental problems, thus removing the need for mastication. Currently there exists little information relating to VFI of various forage sources offered to older horses with compromised dentition. The aim of this study was to investigate dry matter intake (DMI), VFI and consumption rates of three fibre sources, hay (H), DM- 915g/kg, short-chop forage (SF), DM - 860g/kg and soaked fibre pellets (FP), DM - 900g/kg, in veteran horses with poor dentition. SF and FP were both commercially available preparations consisting of high temperature dried grass and alfalfa (SF) and sugar beet and alfalfa (FP).

Materials and methods 12 horses (n=12) of varying breeds were recruited to the cross-over designed trial. 9 of the 12 horses were classified by a qualified equine dentist as having poor dentition (excessive wear, and/or missing teeth), which, in turn, compromised fibre intake. All horses were turned out for 10 hours per 24 hour period, and feeds were offered to all animals upon return to the stable. Previous studies investigating the effects of bulk density and composition of feed on VFI, consumption rates and chew rates, established that test meals of 1kg were adequate (Lowe *et al.*, 2004). However, preliminary studies carried out in this instance found that horses with poor dentition could become over faced, and therefore the test meal was reduced to 0.5kg. Food not consumed within 45 minutes was deemed to have been rejected. The inclusion of the test meal into the normal ration provision, was taken into account, and the subsequent remaining "normal" ration (hay) was adjusted accordingly, thus avoiding overall reduced intake and compromised welfare. Daily rations were formulated to current NRC guidelines. FP was soaked according to the manufacturers guidelines. Each treatment period consisted of a two-day adaptation phase, during which, the animals were introduced to their new diet, followed by a four-day data collection period, as detailed in Thorne *et al.*, (2005). Upon presentation of the test meals, (offered in 10ltr trug buckets) horses were timed to ascertain intake rates. After 45 minutes any remaining food was weighed, and VFI (fresh weight) and DMI were calculated. The resulting data were analysed by analysis of variance & Kruskal-Wallis using Genstat 8.

Results Very highly significant differences were found in VFI and DMI between all three forages (H, SF, FP), when fed to horses with poor dentition. Consumption time was significantly less for FP, than both H and SF (see table 1). A Kruskal-Wallis analysis was carried on data obtained from the "normal" horses, and found no significant differences in VFI and DMI, but found that again, consumption times for FP was significantly (p<0.05) reduced.

Table 1 Effect of different fibre sources on intake.

	Feeds			Sig	SED
	SF	FP	H		
DMI (g)	311 ^a	406 ^b	137 ^c	***	6.1
VFI (g)	361 ^a	451 ^b	149 ^c	***	6.8
Consumption time (mins) Poor dentition	39.8 ^a	23.2 ^b	41.8 ^a	***	0.48
Consumption time (mins) Normal dentition	10.8 ^a	8.9 ^b	13 ^a	*	1.65

^{a,b,c} Values in the same row, not sharing common superscripts are significantly different (***P<0.001, *P<0.05).

Conclusion The wide variety of fibre sources currently available on the equine feed market allow the horse owner increased flexibility and choice. Although many factors need to be considered when deciding upon a ration, for example, energy requirements, protein requirements etc., when choosing rations for older horses with poor dentition, intake also is of utmost importance. This investigation has shown that horses with compromised dentition show very highly significantly increased consumption times when fed hay or short chopped fibre, compared to soaked pelleted feeds. Such pelleted feeds, also resulted in very highly significant increases in both DMI and VFI, compared to both hay and chopped forage. It is therefore possible to conclude that, when feeding veteran horses with compromised dentition, soaked pelleted feeds should be given preference in the ration over course fibre feeds, thus allowing optimum intake of nutrients.

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Cereals, hay and straw high in selenium by use of selenium enriched fertilizers : effects on selenium status in exercised horses

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Introduction Selenium (Se) is a trace element of importance implicated in many processes such as antioxidant mechanisms, immune response, fertility in males and females, protection factor for cardiovascular diseases and some cancer processes (see review by Rayman 2000). There are mainly two modes of action for Se either by a direct implication of metabolites such as the methylselenol or by the specific effects of selenoproteins in which Se is an essential compound in the active site as selenocysteine. Glutathion peroxidase (GPx), thioredoxine reductase, iodothyronine deionidase are some of the selenoproteins. Most feedstuffs produced in Western Europe are low in Se. So, Se has to be supplemented in animal diets. This could be done either on an organic or on an inorganic form. Selenate contained in fertilizers is naturally transformed in organic forms such as selenomethionine by the plants. The aim of the present work was to assess, in exercised horses, the effects on the antioxidant status of feedstuffs produced with Se enriched fertilizers.

Materials and methods Four g Se/ha were applied with the third nitrogen application on winter barley and on spelt fields. Similarly 3g Se/ha were applied with the fertilizer in a pasture for hay production. Those feedstuffs were incorporated in a horses ration containing 57% compound feedstuff and 43% roughage. The compound feedstuff was made of rolled barley (475g/hg), whole spelt (475g/kg), molasses (30g/kg) and mineral mixture free of Se (20g/kg). A diet made of the similar ingredients but grown without Se in the fertilizer was used as control. Six adult horses trained four days a week were used in a cross-over design with two periods of eight weeks preceded by transition periods of two weeks. Blood samples were taken on the jugular vein on 4 occasions before the morning meal.

Results The Se contents in the control and the Se diets were 63 and 297 µg/kg DM. A content of 297 µg/kg DM was three times higher than 100 which is the NRC (2007) requirements for horses. It was much lower than 2000 recognised as maximum tolerable concentration. The average Se content in plasma was significantly increased with the Se supplemented diet (Table 1). It was low and did not change with time in the control group (Figure 1). By contrast, it steadily increased with supplementation. There were no significant differences on the averages of GPx activity in the red cells but one has to note a time effect for the GPx activity which was significantly increased ($P < 0.01$) on the end of the period in the Se group (Figure 2). The delayed effect has to be related to a long half live of the red cells. Total and oxidized glutathions were either significantly or numerically higher in the Se groups as compared to the control group. The differences were interpreted as a sparing effect of Se allowing glutathion to be more available in order to prevent peroxidation. There were no effects of Se supplementation on lipid peroxides.

Table 1 GPx activity and concentrations of metabolites implicated in the antioxidant reactions.

	Control diet	Se diet	SEM	P<F
Plasma Se (µg/l)	111.9	142.7	3.4	0.001
Red cells GPx (IU/g Hb)	207.3	213.8	22.3	0.579
Total glutathion (µmol/l)	1013.8	1148.3	90.4	0.044
Oxidized glutathion (µmol/l)	7.7	11.2	3.4	0.350
Lipid peroxides (µmol/l)	85.8	89.0	19.9	0.748

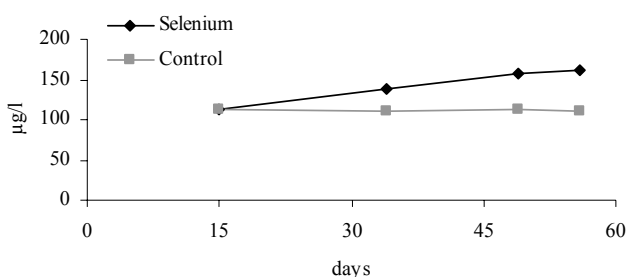


Figure 1 Changes in plasma Se concentration

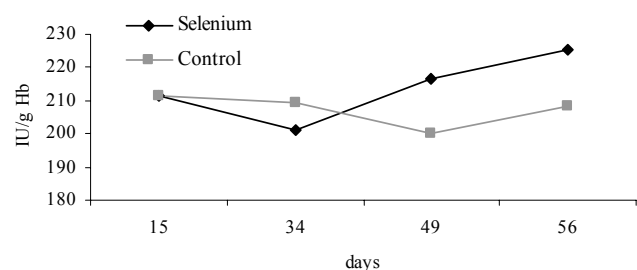


Figure 2 Changes in red cells GPx activity

Conclusion The use of Se enriched fertilizers is a good technique to improve the Se content of locally produced feedstuffs which will be beneficially used in horses diets with, as results, an improvement of the antioxidant status.

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The effect of a supplementary probiotic on faecal *Lactobacilli* count and faecal pH of horses

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Introduction Probiotics have been found to stop pathogenic bacteria from colonising and flourishing in the gut and thus could potentially prevent illness and even death (Kyriakis *et al.*, 1999). The organisms contained within probiotics are also suggested to be able to break down food into a higher number of volatile fatty acids, which increases energy production for the horse (Yuyama *et al.*, 2004). Probiotics are however not very heavily researched in horses and the research which is present is either focused on neonates, young foals or older horses (Kumagai *et al.* 2004). *Lactobacillus* spp. are some of the most commonly used bacteria in equine probiotic supplements as they have shown good success in human based trials and seem to induce positive effects which would be useful to the horse (Laake *et al.*, 2005). Research is however required in this area to investigate if *Lactobacillus* spp. can survive the acidic and bile environments of the equine gastrointestinal tract to a sufficient level to induce beneficial effects. The aims of this study were to discover if feeding a mixed culture probiotic supplement would alter the *Lactobacillus* spp. count in horses and whether this effect is similar in horses of different ages.

Materials and methods Six clinically healthy in foal thoroughbred mares (mean age 12.8 years \pm s.d. 2.48) and six clinically healthy yearling thoroughbreds (mean age 10.3 months \pm s.d. 0.82) were all kept in the same routine for the duration of the study. Faecal samples were taken, (as Weese *et al.*, 2003), two days prior to the start of supplementation and were refrigerated at 4 °C within 20 minutes of collection (Kanamori *et al.*, 2004). The samples were pH tested and then underwent a two fold serial dilution and were cultured anaerobically on Rogosa agar at 37°C for 18 hours. The number of *Lactobacillus* spp. colonies was then counted. Horses were then fed a commercially available supplement containing *Bacillus subtilis*, *Saccharomyces cerevisiae*, *Lactobacillus acidophilus*, *Enterococcus faecium*, vitamins A, D3, E, B6, B12 and C. The manufacturers' recommended dosage rates were 10-15 g for adult horses and 5-10g for youngstock, daily. The mares were therefore given 15g of the oral probiotic supplement and the yearlings were fed 10g of the supplement, via an oral syringe, daily. Faecal samples were collected on days 2, 4, 6, 8, 10 and 12 of supplement administration period and analysed as stated previously. A Wilcoxon signed rank test, Mann Whitney u test, Friedman's rank test for K correlated samples and a Spearman Rank Order Correlation were used to analyse the resulting data.

Results No significant differences were observed between the pH of the mares' faeces ($Z=-0.624$, $p=0.589$) and the youngstock' faeces, nor did the *Lactobacillus* spp. count differ between these two groups ($Z=-1.281$, $p=0.020$), therefore the two groups were treated as a combined sample with results summarised in table one. A significant negative correlation was found between pH and *Lactobacillus* spp. ($R_s=-0.539$, $p=0.000$) with a line of regression of $pH=6.89563 - 0.00224$ *Lactobacillus* spp. count.

Table 1 The effect of supplementation on faecal pH and *Lactobacillus* spp. count (mean \pm s.d.)

	Day 0	Day 14	p	Significant difference between days
pH	6.68 ± 0.26	6.08 ± 0.35	0.027	0,2 > 4,6 > 8,10,12,14
<i>Lactobacillus</i> spp. count	113.08 ± 33.30	287.92 ± 74.53	0.019	0 < 2 < 4,6,8,10 < 12,14

Conclusion Both yearling and mature horses responded to supplementation with the oral probiotic supplement in a similar fashion throughout this experiment. The decrease in pH and increased *Lactobacillus* spp. count in both groups of horses was a very positive result and showed that lactic acid producing bacteria were colonising the intestinal tract. This study demonstrates that feeding probiotics as a daily supplement will increase these beneficial bacteria in the gastrointestinal tract of horses which could be beneficial in the prevention of life threatening conditions, such as colic and laminitis in the horse.

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Preliminary evaluation of weight estimation equations in sport horse type foals (*Equus caballus*)

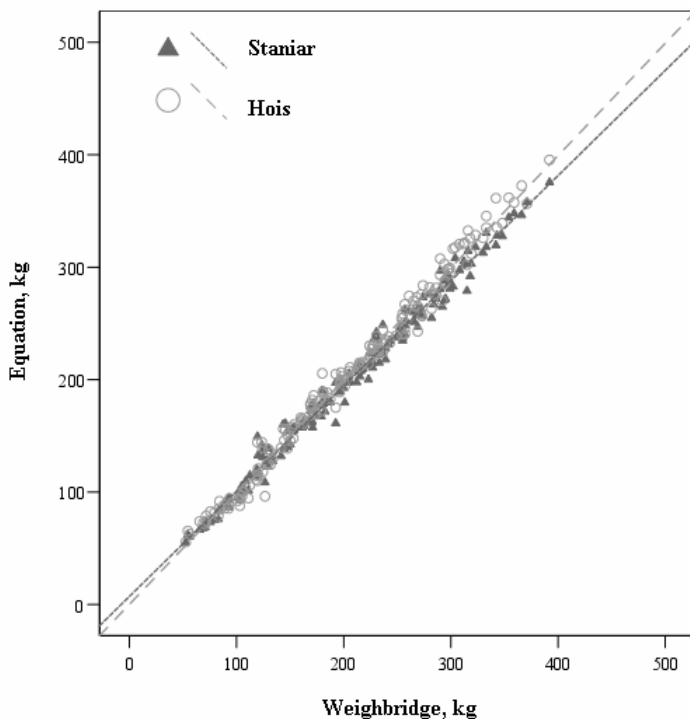
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Introduction Weight estimation in foals is of vital importance for dosing of medication and antihelminthics as well as for assessing nutritional requirements to optimise growth. Calibrated weighbridges are not routinely available and estimates by eye or using weightapes can be inaccurate due to differences between adult and foal body dimensions. This study aimed to assess the applicability to Sport Horse type foals of two body weight estimation equations developed for American Thoroughbred (Staniar *et al*, 2004) and Warmblood (Hois *et al*, 2005) foals.

Materials and methods Ten foals at Home Farm Stud, Hartpury College, Gloucestershire, were weighed and measured fortnightly from shortly after birth until weaning at around 6 months. Data was then collected monthly until February 2007, when foals were aged between 7 and 11 months. Precision, accuracy and bias of the estimates provided by the two equations were compared by regressing results against actual weight obtained using a calibrated weighbridge.



Results Regression against actual body weight gave an r^2 value of 0.99 for both equations ($p < 0.01$). The Staniar equation was slightly more precise (standard error of the estimate 8.30kg) than the Hois equation (9.01kg). However, Hois equation estimates gave a smaller mean error of 4.08% versus 4.51% using the Staniar equation. Their accuracy was also slightly higher based on our analysis of line of identity plots (Fig 1). This was largely due to a degree of bias evident in the Staniar plot as a tendency to underestimate foal weights above approximately 300kg, such that the slope was less than 1 ($p < 0.01$).

Conclusion The preliminary data suggest that two equations developed for other breeds can be successfully generalised to Sport Horse type foals, allowing accurate weight estimation. The larger number of variables used in the Hois equation (Fig 2 a) results in very accurate estimates, while the speed and simplicity of measurement using the Staniar equation (Fig 2b) make it cost effective in terms of time and labour for estimating weight in youngstock, especially at weights below 300kg.

Fig 1 Line of identity plots for weight estimates using equations

Circumference	Predictive equation
≤225cm	$-143.8 + 0.29 * \text{body circ} + 0.98 * \text{girth circ} + 0.44 * \text{withers height} + 5.24 * \text{Body Condition Score}$
226-310cm	$-328.7 + 0.81 * \text{body circ} + 1.67 * \text{girth circ} + 2.36 * \text{cannon circ} + 0.50 * \text{neck base circ}$
311-365cm	$-626.4 + 1.41 * \text{body circ} + 1.76 * \text{girth circ} + 0.63 * \text{withers height} + 6.0 * \text{cannon circ} + 0.75 * \text{neck base circ} - 1.08 * \text{foreleg length (fetlock to elbow)}$
>365cm	$-1160 + 1.538 * \text{body circ} + 1.336 * \text{girth circ} + 2.594 * \text{withers height} + 6.226 * \text{cannon circ} + 1.487 * \text{neck base circ} + 13.63 * \text{Body Condition Score}$

Figure 2a) Hois conditional equation

Volume $((\text{girth circ}^2 \times \text{body length}) + 4 \times (\text{carpus circ}^2 \times \text{foreleg length})) / 4 \times 3.14 (\pi)$

Weight $\left\{ \begin{array}{l} \text{If volume} < 0.27, = \text{volume} \times 1093 \\ \text{If volume} > 0.27, = (\text{volume} \times 984) + 24 \end{array} \right.$

Figure 2b) Staniar equation

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Varying forage stem length as a behavioural enrichment for stabled horses

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Introduction Many domestic horses are kept in an environment very different from that of free-living horses, consuming a varied ad libitum forage based diet for up to 18 hours of the day (Harris, 1999). Encouraging foraging behaviour, defined by Goodwin *et al.* (2002) to include sniffing, manipulating, biting, chewing or ingesting food, is thought to allow domesticated horses to spend more time eating, approaching the time spent on this activity in free-living horses. The diet of the free-living horse includes a selection of grasses and herbs (Putman *et al.*, 1987) whereas most domestic horses are provided with a single forage diet (Goodwin *et al.*, 2002). In a short term trial Goodwin *et al.* (2002) found that offering more than one source of forage to stabled horses resulted in them spending significantly more time foraging compared to a horse on a single forage diet. This effect was found to continue for longer periods by Thorne *et al.* (2005), with four forages being identified as maximising foraging time and minimising standing time (Dumbell and Tackley, 2007). Storing and using four forages may however be impractical for the single horse owner. The present study aimed to determine whether providing two forages or one forage as two different stem lengths increased foraging time when compared to a single forage.

Materials and methods Ten individually housed horses (aged 6 – 17 years, various breeds) were fed ten forage trial diets. All trial diets were fed on an ad libitum basis for four days. On all diets a total of 6 kg of forage, on a fresh basis, was provided. Horses received an additional concentrate ration, according to individual requirements; for each horse this remained consistent throughout the trial period. A 10 x 10 latin square designed trial was chosen to minimise persistent effects from previous diets. Two forage types, hay (H) and haylage (HL), were sourced and half of each forage type was cut to between 1-3cm in length, short stem, whilst the other half was left at its original, long stem, length of 10-20cm. This resulted in four different forage components being investigated: short stem hay (SSH), long stem hay (LSH), short stem haylage (SSHL) and long stem haylage (LSHL). These were fed individually and in every possible pair combination (3 kgs, on a fresh basis, of each) resulting in the ten trial diets. Subjects had both hay and haylage introduced to their diet in the seven days prior to the start of the data collection to allow microbial populations in the large intestine to adjust and minimise digestive upsets. Behavioural observations were taken by point interval sampling every 5 minutes for 30 minutes at 0, 1.5, 3 and 4.5 hours after diet introduction on days 1, 2 and 4. Water was provided on an ad libitum basis throughout the period of data collection. Data were subjected to a General Linear Model Analysis of Variance and Tukeys HSD post hoc tests.

Results Foraging time was significantly increased (all $p < 0.001$) and looking around and moving behaviours were significantly decreased on all of the two forage diets compared to the one forage diets (all $p < 0.01$), as shown in table 1.

Table 1 Effect of trial diets on observed behaviours (mean \pm s.d.)

Trial Diet	Foraging	Look	Move	Other
LSH	34.8 \pm 1.48	13.8 \pm 1.55	12.1 \pm 0.88	23.3 \pm 0.90
LSHL	36.0 \pm 2.05	12.9 \pm 0.99	11.9 \pm 0.99	23.2 \pm 1.11
SSH	33.3 \pm 1.49	15.1 \pm .099	12.9 \pm 0.99	22.7 \pm 1.27
SSHL	34.9 \pm 1.45	14.8 \pm 1.40	12.1 \pm 1.45	22.2 \pm 1.32
LSH & LSHL	49.0 \pm 4.29	8.7 \pm 2.91	7.2 \pm 1.32	19.1 \pm 1.49
LSH & SSH	55.5 \pm 4.55	7.3 \pm 3.20	5.2 \pm 1.48	16.0 \pm 0.98
LSH & SSLH	50.6 \pm 5.25	8.5 \pm 3.03	6.7 \pm 2.58	18.2 \pm 1.40
LSHL & SSLH	57.0 \pm 4.57	7.1 \pm 3.35	4.9 \pm 1.29	15.0 \pm 1.65
LSH & SSLH	50.1 \pm 5.36	8.1 \pm 3.28	7.2 \pm 2.10	18.6 \pm 1.47
SSH & LSHL	52.6 \pm 5.06	8.4 \pm 3.27	6.2 \pm 0.92	16.9 \pm 1.67
p	0.000	0.000	0.000	0.000

Conclusion Feeding two forage components produced a positive effect on stabled horses' behaviour when compared to horses fed a single forage component diet. Surprisingly the combination of long and short stem delivery of a single forage type increased foraging time and decreased looking around and standing behaviours more than feeding two different forage types. This presents a cost effective means of improving horses' welfare which may be particularly appealing to the one horse owner. Increased research to understand the factors that stimulate foraging behaviour in horses is required.

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Investigation to determine whether a preference of forage types exists within horses

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Introduction Horses have evolved to naturally eat a mixed forage based diet (Thorne *et al.*, 2005). Domestication has led to restricted access to pasture and controlled monotonous diets unnatural to the horse, which may lead to Equine Gastric Ulcer Syndrome or Exertional Rhabdomyolysis (Azorturia), as well as behavioural ramifications. The discovery of a highly preferred forage type, more natural to the horse, may enrich the monotonous restricted environment and reduce the high amount of concentrates fed (Goodwin *et al.*, 2007).

There is relatively little published information about forage preference within the domestic horses, with major controversy on the issue of why preferences occur. Theories of forage selection include nutritive quality, previous experience, and palatability or forage enrichment. The present investigation aimed to discover if preferences do exist within horses towards particular forage types.

Materials and methods A total of seven horses (varying in sex, age, breed and weight) were subjected to a preference test in order to assess differences in preference for four commercially available forage types (ryegrass, high fibre (fibre 34-40% and lower protein levels 7-10%), alfalfa and timothy). All horses continued to receive their diet of haylage and low energy concentrate at 80:20 ratios throughout the duration of the study. The test forage was presented simultaneously in varying orders twice daily (Figure 1). Observations of 15 minutes (morning and night) were conducted for five consecutive days, using a wireless video camera, upon the feeding duration, frequency of visits, number of bites, amount of feed consumed, and the number of times forage was smelt but left in favour for another for each forage type. The Anderson-Darling test was used to test for normality and results were then subjected to a General Linear Model Analysis of Variance and Tukeys HSD post hoc tests.

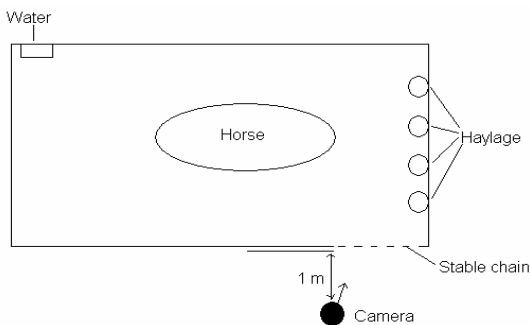


Figure 1 Experimental layout

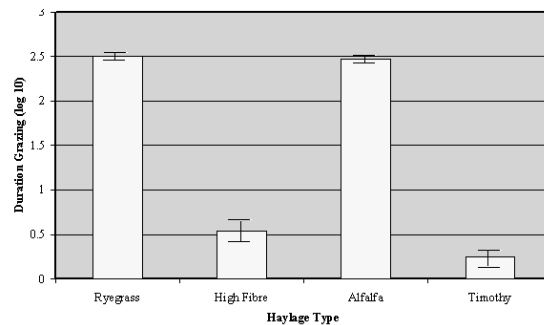


Figure 2 Interval plot of log of duration feeding in relation to forage preference. Bars are one standard error from the mean

Results There was a significant difference ($P < 0.001$) in the frequency of visits and duration of feeding observed with different forages (Figure 2). A preference rank was found for ryegrass, alfalfa, high fibre and timothy (most preferred to least preferred respectively). Individual horses had a highly significant effect on preference for different forage type ($P < 0.001$). The time of day and experimental day did not affect results ($P > 0.05$).

Conclusions Horses demonstrated preferences for particular forages but also were found to feed a variety of forages other than the highly preferred forage. The use of a variety of forages including those highly preferred may reduce wastage and costs, a reduction may also be made in the amount of concentrate fed, whilst potentially minimising digestive problems and promoting natural foraging behaviour. This study therefore supports previous research of this nature.

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Developing research into practice - Extension at Grassland Development Centre, IGER

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The Grassland Development Centre is a dedicated extension team embedded in a Research Institute whose remit spans from the cell to the landscape, from plant breeding to animal metabolomics, and from empirical blue sky science to applied farming systems research. The team seeks to bridge the gap between the researcher and potential end users and to develop research outcomes into commercial land use context with stakeholders.

GDC works predominantly in Wales where the majority of farms are livestock based systems, all dealing with increasing pressures of maintaining economic viability and responding to Welsh Assembly policy initiatives. Developing and using IGER research outputs to help tackle some of the challenges of farming is key to the GDC activities. The team is also actively involved in 'near market' applied research projects with Research - Scientist colleagues to improve the application and dissemination research findings. Working with many partners across Wales and commercial partners, who directly benefit from plant breeding and screening research at IGER, extension methods include demonstration farms, discussion groups, developing factsheets that incorporate the latest research findings and capitalising on the links within the Institute. Although demonstration farms are successful at disseminating 'best practice' to a wide audience, the 'innovators' and the 'early adopter's in the farming community are the stakeholders in the extension process with whom GDC work, that enable real progress to be made in new management methods and adoption of novel technologies or in novel ways. Facilitation skills at several levels are essential for communication to be effective. GDC works with common themes across all livestock sectors from grass and silage quality initiatives, optimising use of on farm nutrients to options for alternative forage crops and possibilities for incorporating energy crops.

Agri -environment research activity to guide government policy in the future, is yielding useful information on semi natural rough grazing and management practices to meet both the desired biodiversity vegetation and to minimise any detrimental impact on animal performance. As this information emerges, and during discussions as part of GDC's work for the Welsh Assembly government running training courses for Tir Gofal agri -environment agreement holders, optimal management methods are being drawn up without compromise to 'prescriptions' of the scheme. Feedback from these training sessions indicates that progress is possible with this type of dialogue which encompasses the principles adopted by GDC of facilitating information transfer in all directions to develop new practices.

Dairy - Despite recent improvements in milk price, there are still great pressures on dairy farmers to minimize their costs of production - and a key aspect of this is to take advantage of a low cost, grass based, systems. To achieve good grass growth rates, high quality forage and high levels of forage utilisation, dairy farmers need access to a wide range of information. IGER research on managing soil structure and soil nutrients, on efficient use of slurry and on managing and utilising high clover/ryegrass content swards all have a vital role to play in cost effective dairy production. However, even the outcomes from very applied research need modification before they can be successfully integrated into a commercial farm. The role of the GDC extension team is to highlight the key principles of the science and to help develop a flexible farm scale approach to use that science effectively. Increasingly, the environmental criteria of dairy farms is coming under scrutiny and a key role for GDC is to help dairy farmers embrace 'greener' farming practices without compromising business performance. Improving slurry usage, combined with reductions in fertiliser and strategies for minimising gaseous losses are key areas of interest on dairy farms as NVZ's expand and milk buyers become more 'carbon aware'.

Organic-Working with Organic Centre Wales, GDC has been instrumental in developing on farm strategies to help organic farmers meet the 100% organic feed requirement. These initiatives include the appropriate use of forage crops in sound rotation; from brassicas to chicory and to highlight their role in an organic system alongside the rapidly increasing use of red clover leys for forage and for fertility building for 'cash' crops of cereals or roots. Building on research work on nutrient budgeting and fertility building at IGER, GDC has also developed with the Researcher, farmer -friendly farm gate nutrient budgeting methods. By encouraging discussion group members to supply information ahead of the meeting, it is possible to compare and contrast the results from a range of real farms within the group, stimulating a wide range of discussion and improving the understanding and importance of nutrient recycling within the farm gate.

Beef & sheep-Pressure to meet market requirements for extended season red meat at minimal cost has likewise prompted beef and sheep farmers to look for cost cutting alternatives to compound feeds and fertilisers. Across demonstration farms and discussion groups, GDC has been exploring the options, mindful of cross compliance issues including animal performance.

Energy crops- Successful integration of these into a Welsh livestock farming system remains a challenge but one that GDC is tackling at several levels. The UK's biomass industry is developing rapidly and it is important that farmers are able to take advantage of the new business opportunities that it brings. Whilst Biomass is unlikely to completely replace livestock farming in areas like Wales, there is scope for integration into traditional grassland farms - to supply either the grower's energy needs or for sale to a local end-user. Miscanthus and SRC willow are well suited to provide high quality feedstock to a growing number of biomass consumers at a range of scales - but those entering this developing industry need technical support. Working through the 'Willow for Wales' project and backed up by a biorenewables science team, GDC can provide agronomic and technical information to both growers and end users. Capitalising on the Secretariat of the Federation of Welsh Grassland Societies' hosted by GDC, the recent annual conference featured the role of energy crops on livestock farms.

EBLEX Better Returns Programme

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Introduction

The objective of the English Beef and Lamb Executive (EBLEX) is to create and maintain a sustainable and competitive beef and lamb sector. Eblex delivers a range of services and information to help farming and red meat chain businesses improve their competitiveness and product quality. Our national on farm technology transfer activities are delivered on a regional basis through the EBLEX Better Returns Programmes for Beef and Sheep, we demonstrate best practice using the latest technology. Enabling producers to access best management practices and improve their skills is critical to ensuring the future financial viability of the sector. In addition producers who are truly getting the best from their animals in terms of efficient feeding, breeding and timely marketing are playing their part in helping address the twin challenges of climate change and better rural landscape management.

EBLEX are encouraging rural sustainability through livestock knowledge transfer partnerships, and we aim to

- Improve the skill base-across technical, management and marketing sectors.
- Technology transfer and innovation- to improve competitiveness.
- Increasing business productivity and profitability.
- Contributing to a sustainable future for livestock production and rural areas.

The Better Returns Programme

Eblex is in a unique position to assess the needs of the beef and lamb sectors and gears its activities to be highly effective and relevant for the current industry situation. Activity under the Better Returns Programme (BRP), initially for sheep and more latterly for beef, typifies how EBLEX is able to identify work areas most appropriate for producers through knowledge transfer and training initiatives.

The priorities and delivery methods for the BRP have been established by taking into account historical and recent research, financial information and broad-based industry consultation. Guidance is provided by industry steering groups that combine the practical viewpoint of farmers with the wider perspective of supply chain expertise.

BRP priorities and methods of delivery are constantly monitored through an event feedback mechanism. There is also great value in using delivery formats that are both practical and are presented by hands-on experts in their respective fields.

Beyond the events, the BRP provides on-going technical information for enrolled farmers in a variety of ways, including printed materials, the farming media and on-line content.

Sheep Better Returns Programme

The £1.5 million Sheep Better Returns Programme (BRP) was delivered by EBLEX between January 2004 and December 2005 and funded via Defra from the 2003 Sheep National Envelope. During the programme more than 12,000 sheep producers directly participated in BRP training events. There were 363 BRP events held, equivalent to more than three events per week during the two year project. More than 80 of these events included a live butchery demonstration. Two TORA(Theory or Reasoned Action) surveys were carried out with Reading University to assess producer attitudes to the themes championed by BRP. In response to the second TORA survey towards the end of the Defra funding, 82.5% of those who responded were aware of the BRP, and 60% had seen BRP publications. The proportion of sheep producers who were weighing and handling lambs regularly to assess finish had increased significantly (by 12% and 38% respectively), with higher rates of change among those exposed to BRP events and publications (TORA). Of producers that had changed their management practices, 46% had noted an improvement. A survey of sheep breeders by Signet said that Sheep BRP had made a significant impact to increasing the interest amongst commercial farmers to performance recorded rams. Exposure to the BRP through participation in events and access to the programme's literature has led to a slight, though significant, improvement in ram selection behaviour. However, findings demonstrate that the behaviour is not common place and therefore BRP activity continues in order to achieve greater application. Exposure to BRP has led to a significant increase in farmers' understanding of market information.

Above is a brief summary of the delivery over the initial two year period of the Sheep BRP which was funded by Defra. It proved the need for focused and practical delivery of new information and technology to producers. In response to industry demand EBLEX has carried on the core activity of Sheep BRP and is half way through a similar Defra funded initiative for Beef. The BRP Themes focus on :-

- Better breeding through the uptake of recorded animals
- Better feeding and use of forage / grazing management.
- Better fertility
- Better selection to meet market demands
- Better costings and systems of production.

SAC developments in practical Knowledge Transfer

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SAC, with its combined role in research, education and consultancy, is uniquely placed to deliver effective KT to the livestock sector of the UK. The consultancy division provides a wide variety of technical consulting services and basic training for a comprehensive range of clients within the land-based sectors. As well as our own programme of KT events which are linked to our research programmes we deliver KT for levy funded organisations throughout the UK mainly aimed at improving competitiveness and product quality.

SAC have been innovative in the development of the Monitor farms programme –initiated with support from the Scottish Government and local authorities, then taken up by levy funded bodies such as QMS, HGCA and MDC. SAC tenders competitively for these contracts which are overseen by QMS in Scotland and currently has seven of the twelve under contract. The monitor farm approach has been very well supported by the industry, its key strength is that it is participatory –with the local community group taking responsibility to choose the monitor farm and get involved with decision making on the chosen unit. The community group are free to bring in specialists to address farm issues and work with the facilitator (local farm business consultant) to produce an informative programme of 6 farm visits. These plot progress and follow through management decisions that the group have helped make. Key aims are to improve the physical and financial performance, and improve farmers' decision making. Use of the farm recording and benchmarking are central to the groups success as this enables the community group to share information and identify their own farm weakness. The objective is to take the whole group forward not just the monitor farm. Knowing where you are technically is the first step to making change and improvement. Because of the success and publicity monitor farms have achieved there is a pressure from research, industry, and NGO's to use the medium to put across their own messages. The structure however, based on farmers' goals is robust enough to keep the three-year programme on track.

Facilitating a monitor farm requires skills in participative training techniques and SAC has invested in these to improve the quality of the monitor farm experience. Most farmers if asked will admit to already knowing more than they have time to apply- rather than more knowledge- they often need help in making changes and seeing them in operation over a sensible time period in relation to sheep and beef production cycles.

SAC also run many groups and have used one to develop easy care sheep systems. Group dynamics were again based on participative techniques and the group set its own goals that were to 'farm without needing to rely on subsidies'. Easy care sheep systems grew from this group to a mainstream system over seven years. Developments included:

- Refining non – interventionist lambing from experience of leading farmers incorporating results from SAC research into behavioural studies
- Getting on top of labour intensive diseases
- Breeding rams to reduce lambing problems using an 'easicare selection toolbox' developed by the group
- Improving outputs through selection based on ebvs
- Deferred grazing and low cost wintering systems including grazed forage brassicas
- Use of modern sheep handling systems

Progress was impressive and quickly the main technical problems were solved allowing farmers to increase lamb sales towards a target of around 1500 per man per year.

SAC used industry partners in the 'Easicare Initiative' who were willing to develop products that were particularly useful in easy care production systems. This allowed time to be spent producing a definitive system guide. EBLEX and HCC subsequently funded the production of booklets such as 'Target Easier management for Better Returns' and 'Breeding and Managing Easier Kept Sheep'

The Sheep -Easy group was established in 2007 to provide appropriate breeding replacements and introduce new breeding technology with help from Genesis Faraday and Spark awards.

Most information produced from research has little chance of uptake by farmers unless someone takes responsibility for turning it into a practical message. Often it will need developing into a product or system that is financially attractive within the current mix of resource availability and cost. Managing this process is most successful when farmers set the goals.

Leading by example – The Demonstration Farm and Discussion Group Network in Wales

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Hybu Cig Cymru (HCC, Meat Promotion Wales) is widely recognised as the leading vehicle for information and support to the livestock/meat industry in Wales. HCC through the Farming Connect Sheep and Beef Development Programme has established and maintains an extensive, pan-Wales network of Demonstrations Farms and Discussion Groups.

The overall objective of the Demonstration Farm and Discussion Group programme is to enable effective Technology Transfer between farmers, researchers and advisors to improve farm efficiency and profitability in Wales and has a series of aims:

- To increase farmers' awareness of manageable factors affecting their business.
- To identify specific targets and monitor the business to ensure that they are achieved on the demonstration farm.
- To encourage those attending meetings to adopt practices that have been successfully applied on the demonstration farm.
- To encourage a business like approach, collective learning and problem solving.

This underpins the three key strategic aims of Farming Connect (namely promoting business development and awareness; environmental safeguarding and enhancement; and improving market focus and awareness).

Demonstrating Success

To achieve these goals the programme has established 10 demonstration farms across Wales. Each farm must:

- Complete a Farm Business Development plan with SMART objectives.
- Complete a Farm Health Plan in conjunction with their local vet.
- Complete an Environmental Options Review (EOR).
- Undertake two projects per farm, each aimed at demonstrating the cost-benefits of adopting a particular technology on the farm.
- Hold one Open Day and up to five discussion group meetings per year.
- Provide information for at least 3 Newsletters per year.

Each farm therefore features a range of up-to date farming techniques and methodologies which have contributed to their increased efficiency and profitability, a more enhanced market focus, and clear improvements in animal health and welfare. The programme enables the results to be disseminated to an extended network of more than 6000 farmers.

Practical ways to achieve success

Whilst the challenges facing farmers across Wales may be the same, practical approaches to meet these challenges can often be quite different depending on the area. HCC supports the coordination of more than 30 Discussion Groups right across Wales. These Discussion Groups aim to bring together neighbouring/local farmers and to provide a forum in which they can openly discuss key topics which affect them and to share experiences. Local coordination helps to ensure that the programme is tailored to the needs of the group and includes workshops which are given by invited specialists who can discuss practical solutions in a local context. Farm walks and visits to various farms/centres also enable Discussion Group members to see new technologies and developments in action and to see how they could work on their own farms. More than 1000 farmers actively participate in this network and the current programme includes topics such as:

Grassland Management & feed quality	Sheep handling systems
Ram Fertility	Abattoir feedback
Easier management systems	Flock and herd health

Lessons from abroad – the experiences of Farming Connect Scholars who have looked at how alternative approaches in other countries

Technology Transfer in Action

The projects and activities undertaken through this programme enable new technologies and methodologies which have been developed through the pipeline of HCC R&D projects as well as from other mainstream research to be taken forward.

Projects currently being undertaken include:

- Effect of aerating the soil on grass growth
- Effective use of anthelmintics
- Impact of weaning management on lamb and calf performance
- Economic impact of retaining bulls for finishing
- Evaluation of performance of Lleyn lambs carrying the MyoMAX gene
- Use of alternative forages - Red clover and Chicory for finishing lambs
- Use of weed wipes to control thistles whilst maintaining clover
- Evaluation of the cost-effectiveness of using a silage additive on the performance of young cattle
- Using high index rams and selecting replacements on prolificacy, lambing ease and muscularity

Dairy Development Centre – Knowledge Transfer

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The Dairy Development Centre (DDC) is one strand of a multi-faceted resource for the agricultural industry known as Farming Connect. The purpose of the Centre is to facilitate the development of the Welsh dairy industry through the provision of a proactive technology transfer service and market intelligence, working in collaboration with key partners to organise events illustrating best practice techniques and new technologies. The Centre is based around a co-ordinating Development Centre and a network of Development Farms, Demonstration Farms and Discussion Groups.

Background to the Welsh Dairy Industry

There are currently 2,268 producers in Wales (September 2007 Datum Wales), a loss of just under 1,000 producers over the last five years. The average herd size has increased from 67 to 75 between 2002 and 2005 (MDC Datum Wales). The majority of producers are based in the old counties of Dyfed and Clwyd.

Total milk production in Wales represents circa 11% of the UK output. Approximately 80% of milk produced in Wales is processed in Wales, with major concentrations in the old counties of Dyfed & Clwyd. It is therefore evident that the dairy sector has particular impact on the broader economy of Wales in key producing areas.

DDC Knowledge Transfer Work

Development Farms - The DDC supports a network of specialist Development Farms as part of the overall dissemination strategy. There are three of these farms - Gelli Aur College, servicing the South West milk field, Llysfasi College servicing the North East milk field and Glynllifon College servicing the North West milk field. All development farms specialise in different aspects of dairying, namely dairying combined with upland beef & sheep at Llysfasi and Glynllifon and lowland dairying with two herd systems as well as beef & sheep at Gelli Aur. All Welsh dairy farmers are no more than two hours travelling distance from these three locations.

Objectives

- Taking research into practice by demonstration on a commercial scale
- Illustrating sustainable husbandry and management strategies
- Generating sound practical advice in order to facilitate efficiency gains
- Demonstrate good practice to the industry and the community in general with particular emphasis on animal welfare and environmental management
- Providing technical updating to consultants, vets and the further education sector

Demonstration Farms - The DDC supports a network of eight demonstration farms. Each farm has a specific area of expertise and in addition to the Development Farms objectives above, places particular emphasis on:

Objectives

- Demonstrate and evaluate different dairy production systems on a commercial scale.
- Illustrate the integration of environmental initiatives, such as Tir Gofal and Habitat Schemes, into the commercial farm environment.
- Provide a vehicle for collaboration with other development centres to disseminate common messages.
- Build greater awareness of the importance of efficient collaboration along the food chain.

Discussion Groups - The DDC supports dairy discussion groups in Wales as they provide the ideal environment to learn. Discussion group meetings include formal evening with invited speakers, farm walks on group member's farms and visits to other people's farms. As well as supporting independent groups, the DDC created a discussion group network as a support group for the demonstration farms. The frequency of the group meetings vary, but the DDC co-ordinate the activity of the groups, and where necessary allocate a champion to provide specific advice.

Results

Year	No Welsh Dairy Farmers	DDC Event Attendance	% Penetration *
2004 / 05	2,661 (Mar 05)	1,391	52%
2005 / 06	2,497 (Mar 06)	1,089	44%
2006 / 07	2,316 (Mar 07)	1,741	75%

* - Some farmers may attend more than one event

Survey of the exercise workload, physiological issues, monetary value and nutrition of working dogs in New Zealand

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Introduction Although there have been many studies examining the requirements and needs of working dogs, most is either unpublished commercial data, or focuses on racing dogs, such as greyhounds and sled dogs. A substantial number of the world's working dog population are farm dogs used on cattle, dairy or sheep farms. The majority of dogs in New Zealand fall into this category, making them a useful population for examining factors affecting their performance. In this initial study, information from more than 3000 working dogs was obtained by questionnaire to establish an accurate picture of the average working dog and to identify feeding practices and areas that could be improved to ensure health, welfare and longevity.

Materials and methods A questionnaire was sent out to 504 farmers throughout New Zealand. Farms varied in climate, terrain and type of stock carried. Farmers were asked to answer a series of questions relating to the age, health status, work load and feeding practises used with their working dogs. Workload was assessed by how many hours per day the dog was actively working, whether the farm was on flat or hill country, and if the dogs were only required at certain times of year (e.g. sheep mustering before lamb sales). Farmers were asked to put the amount of training required in order to estimate the cost of training each dog. The questionnaires were analysed using the SPSS statistical program (Version 15, Chicago, USA) to calculate averages (\pm standard deviation) for all parameters. This survey forms the basis of a series of trials conducted to improve the nutrition and feeding of working dogs.

Results Table 1 shows the averages of the main parameters identified in the questionnaire.

Table 1 Initial survey results of exercise workload, physiological issues, monetary value and nutrition of working dogs on 504 farms in New Zealand

Parameter	Average	Standard deviation
No. dogs per farm	7	3.7
Farm size (acres)	1196	2563.4
Distance to nearest town (km)	26	21.2
Dog Age (years)	3.8	2.91
Body Weight (kg)	25.9	8.06
Days off work (d/year)	9.2	6.11
Tiredness after normal working day (1-5 scale*)	2.7	0.97
No. dogs euthanased in previous 12 month period	1.2	0.51
Peak working period:		
Dry food (% of total diet)	39.8	24.89
Fallen stock meat (% of total diet)	59.7	24.20
Off-peak working period		
Dry food (% of total diet)	43.5	24.49
Fallen stock meat (% of total diet)	53.8	24.36

*1 = not tired at all, 5 = extremely tired

The survey revealed that the average age of working farm dogs was very low – less than 4 years old. On average, producing a useful working dog takes 18 months, and requires an extra 1 hour in a working day, equating to a total of 374 hours, worth NZ\$5612.50 at a rate of pay of \$15/hour. The data showed that 22 % of farms reported at least one dog that was euthanased in 2006, and 42% were reportedly sick or injured. From the nutrition perspective, the vast majority (97%) were fed once per day. Diets were remarkably consistent between busy and quiet farming periods, with over 50% of the diet being supplied as fallen stock (termed 'home kill' meat in New Zealand) and around 40% as dry commercial food.

Conclusions The low average age indicates a major wastage of animals in this industry, and low payback for the amount of time dedicated to training a young working dog. The cost of training a dog is significant in terms of man hours alone, and represents a major investment for farmers. Improving longevity is important in ensuring payback for this investment. The lack of attention paid to feeding the dogs according to their work requirements may be related to poor life expectancy and injury or illness rate. Further work is continuing to monitor the body condition score of working farm dogs throughout the farming year, and investigate how changes in nutrition and feeding practises can improve work performance, stamina and longevity.

Rapeseed meal as an alternative protein source for culturing common carp (*Cyprinus carpio*)

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Introduction Fish meal based diets are important in fish farming. As with other living organisms, the growth, health and reproduction of fish are dependent on adequate nutrient supply, and feed quality has a significant impact on the quality of fish meat. The aquaculture consumes 70 per cent of the global production of fish oil and 34 per cent of the total fishmeal. It is estimated that the demand for fish meal (FM) for feed will increase even more. This increased demand will surpass the natural fish supply and so it is crucial to find alternative protein sources to reduce pressure on the natural fish ecosystem. It is essential that the alternative sources compare well with the fish by-products and do not harm the fish health and growth. Therefore, this study examined the suitability of UK produced rapeseed meal (RM) as an alternative to FM to prepare nutritious diets to maintain mature fish and their impact on the water quality. Common carp (fish) was selected for their ability to cope with variable situations and for their low rearing cost.

Materials and methods The experiment was conducted in a shed at the Angel of the North fish lakes by using circular tanks and a completely randomised design. Twenty three mature fish with mean live weight of $653\text{g} \pm 38$ (SE) were selected from the ponds and distributed into eight tanks (seven with 500 and one with 250 litres capacity). Due to the variable water capacity seven of these tanks received three fish each whereas the smaller tank received two fish. The tanks were non-circulating without any outlet, so the frequent filtration and continuous air circulation were done by using external filters (Aqua Vital AVEX 1200) and air circulation pumps (Yasunage LP 120H). The tanks were covered with mesh. Four iso-nitrogenous (250g crude protein (CP) /kg DM) coarsely mixed diets were formulated with % CP substitution level of 0 (A), 25 (B), 50 (C) and 75 (D) of FM with RM. Other ingredients were flaked barley, oil (15% of the diet), and mineral and vitamin premix. Each of the diets B, C and D was offered to six fish in two tanks per diet but diet A (control) was offered to five fish in two tanks due to the one smaller tank. Each feed was offered once a day at about 3% of live-weight (LW) and the amount of feed adjusted daily according to feed refusals by the fish as monitored visually. The water quality was checked daily which is presented in another paper of this meeting. Weekly fish LW and water temperatures (Temp) were recorded and statistically analysed to study the effect of diets on LW and Temp at $P < 0.05$ by using Minitab.

Results Although diets showed variations in fish LW and water Temp at different days, these variations were non-significant ($P > 0.05$; Table 1). The fish were unable to grow much in response to most diets except diet D with 75% RM CP for which fish gained 1.2g LW /day compared with other diets where the fish either gained nil or smaller LW over 43 days. While the mature fish of this study were not expected to grow substantially, the small LW gains observed for fish were attributed to the lower than the desirable (23°C) water Temp for fish growth in unusually 'cold' summer of 2007.

Table1 Mean fish live-weights and water temperatures of tanks at different days with standard errors of mean (SEM)

Days	Fish Live Weight (g)				SEM	Water Temperature ($^{\circ}\text{C}$)				SEM
	A	B	C	D		A	B	C	D	
1	690	728	605	610	84	14.2	14.5	14.5	14.0	0.31
8	620	713	596	598	82	14.7	14.7	14.6	14.6	0.53
15	642	758	615	628	85	15.9	15.9	15.9	15.9	0.17
22	672	755	610	661	85	15.4	15.5	15.5	15.2	0.36
29	668	751	605	648	83	14.8	14.9	15.0	15.0	0.20
36	668	743	601	653	77	14.6	14.3	14.6	14.7	0.11
43	680	721	626	663	76	14.8	14.9	15.1	15.0	0.20

Conclusion It appears that rapeseed meal can replace up to 75% of fish meal CP without any adverse effect on fish health and growth. The fish were able to grow better with 75% rapeseed meal CP than 100% fish meal CP even when on coarsely mixed diets and sub-optimal temperatures of $< 23^{\circ}\text{C}$ in summer 2007. Further studies are examining the effect of these diets on the fish meat quality and composition.

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The effect of enclosure design on the behaviour of captive Ring-tailed lemurs (*Lemur catta*).

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Introduction The principal method of assessing a captive animal's welfare is by observing their behaviour and comparing it to its wild relatives (Carlstead, 1996). Enrichment often takes the form of a complex, diverse enclosure where the individual can, for example forage for food, mark its territory and maintain its physical condition which allows the lemurs to exhibit more 'natural' behaviours by exhibiting some control over their environment and thus maintain high welfare standards (Carlstead, 1996). Whilst previous studies suggested visitors resulted in negative behaviours in primates (Chamove *et al.*, 1988), more recent work suggests that the presence of humans within their enclosure is a form of enrichment for ring-tailed lemurs (Simpson, 2004). The aim of the study was to investigate the effect of the 'traditional' versus the larger, more varied walk-through enclosure on the behaviour of ring-tailed lemurs.

Materials and methods The animals were housed at Cotswold Wildlife Park for the duration of the study and comprised of 1 adult male, 3 adult females and 2 juvenile males, however only the adults were studied as the juveniles were in the process of being weaned. The lemurs were individually identified by their physical characteristics such as the patterns of tail banding and face markings, as well as size and coat colour. On five consecutive days in the traditional enclosure, behaviour was assessed by observations during each of the feeding times (approximately 0900, 1200 and 1500) using the scan sampling technique. Where scan sampling involves recording each individual's behaviour every minute for a period of 30 minutes. In addition, each individual was observed for a focal sampling period of 30 minutes where an individual's behaviour was recorded continuously. This regime of observations was repeated following a 7-day acclimatization period to the walk-through enclosure. A total of 15 behaviours were recorded but only resting, locomotion, vocalisation, fighting, foraging and feeding are presented here. The percentage time spent performing each observed behaviour during the focal sampling period was calculated and compared between the enclosures. The difference between the times spent performing each observed behaviour in the two enclosure types were assessed with a one-sample Wilcoxon test in Minitab v15.

Results In the walk-through enclosure, the lemurs were more active with increased locomotion (Table 1; $P < 0.001$) and foraging ($P < 0.005$) behaviours and less time spent resting ($P < 0.001$). In addition, the time spent feeding was less in the new enclosure ($P < 0.001$) and the number of vocalisations decreased ($P < 0.01$). There were less aggressive interactions as indicated by a decrease in the time lemurs spent fighting ($P < 0.005$).

Table 1 Percentage time spent for each observed behaviour

Behaviour	Traditional enclosure	Walk-through enclosure	Significance
Locomotion	2.48±0.89	4.66±0.89	$P < 0.001$
Foraging	1.54±0.86	2.70±0.66	$P < 0.005$
Resting (non-sleep)	17.62±3.16	3.26±1.11	$P < 0.001$
Feeding	14.16±3.74	3.83±1.12	$P < 0.001$
Vocalisations	1.33±0.94	0.04±0.04	$P < 0.01$
Fighting	0.52±0.27	0.02±0.01	$P < 0.005$

Conclusions This investigation demonstrated that the walk-through enclosure is a more enriched environment for ring tailed lemurs. In contrast to Spinelli and Markowitz (1985) this study has shown that in the larger walk through enclosure, which had been designed to be more natural looking, activity levels were increased. The authors believe that the lemurs experienced higher welfare from the enrichment, were healthier physically from increased locomotion and displayed more 'natural' feeding behaviour – both in terms of the time and the reduction in vocalisations which is associated with begging (Carlstead, 1996). Lemurs released from captivity display behaviours of increased agility and foraging behaviour which is similar to those who have always lived in the wild (Keith-Lucas *et al.*, 1999), indicating that the lemurs in this study were able to mimic, to a lesser extent, this release. This study indicates that the success of the walk-through enclosure at Blackpool (Webster, 2000) has been mirrored by the one at Cotswold Wildlife Park however further work is required with a larger group of animals to confirm our observations.

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Some hair characteristics of camels in two provinces of Iran

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Introduction The camel carries a mixed fleece similar to the Cashmere goat and renowned as specially hair or luxury fibres. The outer hair of animal is very coarse, tough, and wiry and inner coat has soft fine down fibres that give a valuable product known as camel's hair wool. Coarse wool and hair is often used for the manufacture of tent cloth, blankets, rope and cloaks. In Iran, the fine down hairs of the inner coat of the camel is usually gathered to make special clothing for men, such as that carrying the 'Aba' name. The young animals yield about 3kg of raw wool while older animals yield only 1-2kg. The hair colour is generally brown, varying from deep chocolate and almost black, through reds, rusts, and fawns to almost white in some types. The objective of this study was to evaluate the camel fibre according to region and age and such information for improving their quality during processing.

Materials and methods Hair samples were taken from 35 and 89 camels, aged 2 to 20 years. (in four age groups) that were managed at Astanghods station in Mashhad city in northeastern Iran, and Yazd province in the central part of Iran., A visual subjective test was first used to separate the inner and outer coat and then the separated inner coat was used for determining the medullated fibres. Almeter and FDA instruments were used to determine the down fibre diameter and Hauteur and Barbe lengths (respective lengths of fibres calculated from the proportion by titre and mass of fibres in slivers).. The wool scouring yield was determined by washing the greasy raw fibres using anionic soap and sodium carbonate with hot water (45-50°C). Data were analysed by multivariate and general liner models (GLM) using the SAS software package.

Results Analysis of variance indicated a significant ($P<0.01$) effect of age and regions on staple length, fibre diameter and medullated percentage, while the effect of sex was not significant. A significant ($P<0.001$) difference was found between medullated fibre of mid-side and under-hump samples. Young camels had significantly ($P<0.05$) longer and finer fibres than the older camels. It has been cited in the literature that genetics and age can significantly affect the fibre characteristics (Banamali, 2000. Beknazarov, 1982 Champak, 2001); such results were also obtained in this study. The results also illustrate that fractionation of camel hair according to age and region can improve processing and supply to market.

Table 1 Difference of least squares means and standard errors for one hump Iranian camel hair characteristics

S.O.V	No	staple length (cm)	Fibre length(mm) Hauteur	Barbe	Diameter (μ m)	Inner Coat (%)	Non Medulla Fibres (%)	Shrinkage (%)
Region								
1	89	4.58 ^b (0.2)	30.0 (1.4)	46.4 (2.2)	23.3 ^b (0.6)	77.1 (2.3)	55.1 ^a (2.9)	22.7 ^b (1.2)
2	35	5.3 ^a (0.3)	32 (1.8)	51.8 (2.8)	26.9 ^a (0.8)	75.5 (2.8)	35.4 ^b (3.6)	34.6 ^a (1.5)
Sex								
Male	9	4.7 (0.4)	30.3 (2.5)	47.8 (3.9)	25.7 (1)	75.9 (4)	46.7 (5.1)	28.1 (2.2)
Female	115	5.1 (0.1)	31.7 (0.9)	50.4 (1.3)	24.6 (0.4)	76.7 (1.3)	43.7 (1.7)	29.2 (0.7)
Age (year)								
2-6	46	5.9 ^a (0.3)	31.9 (1.6)	56 ^a (2.5)	23 ^c (0.7)	72.3 (2.6)	48 ^a (3.3)	24.2 ^b (1.4)
7-10	41	4.6 ^b (0.3)	31.9 (1.7)	48 ^a (2.6)	24 ^b (0.7)	74.6 (2.6)	46.8 ^a (3.6)	27.4 ^{ab} (1.5)
11-14	23	4.7 ^b (0.3)	29.4 (1.9)	45 ^b (3)	27 ^a (0.8)	79.2 (3)	41.1 ^b (3)	28.5 ^a (2)
>14	14	4.6 ^b (0.4)	30.0 (2.5)	47.5 ^{ab} (4)	26 ^{ab} (1)	79.2 (4)	45 ^{ab} (5)	27.1 ^{ab} (2.1)

*. a, b, c: Means with different superscripts in the same column differ significantly ($P<0.05$, 0.01 and 0.001).

Conclusions The results illustrate that fractionation of camel hair according to age grouping (young camel separated from adult) can improve supply to market and manufacture processing.

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Effect of rapeseed meal substitution on the water quality of fish tanks containing common carp (*Cyprinus carpio*)

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Introduction Global aquaculture production has been growing at about 8% per year compensating for the stagnation of fisheries, and it is likely to become the main source of aquatic food for humans as demands continue to grow. This development in aquaculture is predicted to replace fisheries as intensive animal production has replaced the land based hunting. To satisfy this predicted demand plant based alternatives to fish meal (FM), the main dietary ingredient for fish production, will be needed. However, the use of such alternatives may affect the water quality which determines the fish health and production. This study hypothesized that replacement of FM with rapeseed meal (RM) as major sources of crude protein (CP) would not affect the water quality of fish tanks inhabited by common carp (fish).

Materials and methods The experiment was undertaken in a shed at Angel of the North fish lakes by using circular tanks and a completely randomized design. Twenty three mature fish with mean live weight of $653\text{g} \pm 38$ (SE) were selected from the ponds and distributed into 8 tanks (7 with 500 and 1 with 250 litres capacity). Due to the variable water capacity 7 of these tanks received 3 fish each whereas the smaller tank received 2 fish. The tanks were non-circulating without any outlet so the frequent filtration and continuous air circulation were done by using external filters (Aqua Vital AVEX 1200) and air circulation pumps (Yasunage LP 120H). Tanks were covered with mesh. Four isonitrogenous (250g crude protein (CP) /kg DM) coarsely mixed diets were formulated with the % CP substitution level of 0 (A), 25 (B), 50 (C) and 75 (D) of FM with RM. Other ingredients were flaked barley, oil (15%), and mineral & vitamin premix. Each of the diets B, C and D was offered to 6 fish in 2 tanks per diet but diet A (control) was offered to 5 fish in 2 tanks due to 1 smaller tank. Each feed was given once a day at about 3% /body weight and the amount of feed adjusted daily according to the feed refusals by the fish as monitored visually. The water quality parameters including temperature (top, middle & bottom of each tank), dissolved oxygen, and pH were recorded on a daily basis for six weeks of this study. . There were negligible differences in daily observations and hence the observations at only the start, mid and end of each week were statistically analysed to study the effect of these diets on the water quality by using Minitab software. The effects of treatments on water quality parameters were declared significant if $P < 0.05$.

Results It was observed that during the whole experimental period there was no effect of the treatments for any of the water quality parameters. The present study showed that the water quality parameters i.e. dissolved oxygen and pH, were within the normal range of water quality criteria for aquaculture (Table 1). In this study, water ammonia levels ranged between 0-0.17 mg/L which was much below the upper limit of 2 mg/L that carp (ammonia tolerant species) can tolerate to maintain health and production. It was observed that the replacement of fish meal with rapeseed meal did not adversely affect the water quality of fish tanks and so it should be acceptable as an alternative protein source to formulate fish diets.

Table 1 Main effects of different diets (A, B, C and D) on the mean values of various parameters

Ammonia (mg/L) Days	SEM				Dissolved O ₂ (mg/L)				pH				SEM		
	A	B	C	D	A	B	C	D	A	B	C	D			
1	0.17	0.09	0.11	0.09	0.03	7.2	7.4	7.6	7.5	0.17	7.6	7.7	7.7	7.6	0.07
8	0.09	0.12	0.12	0.08	0.03	7.8	7.8	7.9	7.8	0.10	7.7	7.6	7.6	7.6	0.06
15	0.16	0.09	0.13	0.06	0.05	7.6	7.7	7.6	7.9	0.18	7.6	7.6	7.6	7.6	0.05
22	0.11	0.05	0.03	0.00	0.03	7.4	7.4	7.4	7.5	0.16	7.6	7.6	7.5	7.6	0.07
29	0.16	0.12	0.17	0.12	0.03	7.7	7.4	7.5	7.7	0.08	7.6	7.6	7.6	7.6	0.07
36	0.16	0.08	0.09	0.08	0.02	7.3	7.6	7.2	7.1	0.12	7.6	7.8	7.7	7.7	0.06
43	ND	ND	ND	ND	ND	7.2	7.2	7.4	7.1	0.10	7.6	7.6	7.6	7.6	0.07

SEM = standard errors of mean; ND=not determined

Conclusion It appears that rapeseed based diets did not harm the water quality of fish tanks. Therefore, rapeseed meal could be used as an alternative protein source for preparing nutritious diets for Carp production.

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Preliminary investigation of tuber coxae - tuber calcanei length in selected groups of event horses

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Introduction Holmstrom *et al.* (1990) highlighted that conformation is regarded as indicative of performance and soundness. However little research has been devoted to confirming the relationship between conformational characteristics and performance. Today most conformational assessment is based on a subjective approach (Holmstrom *et al.* 1990). Several authors have stressed the most important part of the ridden horses conformation are its hind limbs (Holmstrom *et al.* 1990). Length between tuber coxae and tuber calcanei (point of hip to point of hock) is regarded by assessor and trainers as an important reflection of the power generated within the hind limb. The belief is that, the longer this length the greater the power generated and potentially the higher level of performance attained. However there is little scientific evidence to verify this. The stated null hypothesis is tuber coxae to tuber calcanei length does not differ significantly in four groups of event horses defined by level of performance.

Materials and method Data was collated from 48 event horses defined by four levels of performance, Pre-Novice (PN) n=16, Novice (N) n=10, Intermediate (I) n=10, Advanced (A) n=11. Measurements were taken directly on each animal from the tuber coxae to the tuber calcanei. Both these points are readily identifiable landmarks on the horse. All measurements were taken by the same individual. Descriptive statistics and measures of dispersion were returned for each discrete group. Performance level data was interrogated via one-way analysis of variance; further Post hoc multiple comparison tests, Least Significant Differences were applied to the data set. All horses measured were at least five years old and had therefore reach physical maturity. Additionally analysis of variance was undertaken for horse height at withers between groups.

Results Table 1 returns the descriptive statistics and measures of dispersion for each discrete performance level group. Analysis of variance via performance level identifying a significant effect ($f=2.986$, $df=47$, $P<0.05$), further post hoc analysis highlighted a significant difference between the advanced group and pre novice group ($P<0.05$) and intermediate group ($P<0.05$). Indications of a trend were evidence between advanced and novice ($p=0.057$) and intermediate and novice ($p=0.057$). No significant differences between wither height and group were observed.

Figure 1 Image of measured reference points - tuber coxae - tuber calcanei length



Table 1 Descriptive statistics for tuber coxae - tuber calcanei length (cm) by level of performance

Group	Mean	Min	Max	S.Dev	s.e.m	Skew
PN	83.87	70.25	94.42	6.374	1.593	-0.252
N	83.99	75.79	93.72	6.225	1.968	0.425
I	88.67	82.20	97.94	4.499	1.356	0.552
A	88.68	82.21	95.88	4.059	1.224	0.079

Conclusions It is evident from this study that an effect relating to level of performance is apparent. The mean tuber coxae - tuber calcanei length for pre novice and novice horses is virtually identical as is the length for intermediate and advanced groups. Essentially there is nearly a 5cm mean difference in tuber coxae - tuber calcanei length between those horses competing at novice and below and those competing at intermediate and above. The work confirms the previously subjective assertion that longer tuber coxae - tuber calcanei length may indicate a higher level of performance. Both horse age and height were not a significant effect within this study; however the number of horses studied within this study is small. A further study on a larger number of animals is required before any firm recommendations can be made to aid objective selection processes.

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The investigation of genetic variation in Mazandaran buffalo population using microsatellite markers

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Introduction Iranian buffalo have a wide geographical distribution. Buffalo production is an important livestock sector, as a source of milk and meat. Knowledge of the genetic population of the buffalo is one of the main steps in breed conservation programmes and may have implications for future breeding strategies. Microsatellites are now widely used since they are numerous, randomly distributed in the genome, highly polymorphic and show co-dominance inheritance (Hillel *et al.*, 2003). The objective of the present study is to determine and characterise the genetic diversity and the genetic structure of the Mazandaran buffalo population by using 13 polymorphic microsatellite markers.

Materials and methods Blood samples were collected from different parts of the Mazandaran province according to the size of the herds, geographical distance and distribution. Genomic DNA was extracted, using modified salting out method. Agarose gel was used to determine the qualification of DNA. Microsatellites were amplified in a reaction mix containing 1 unit of Taq DNA polymerase, 3 mm MgCl₂, 200 µm of each dNTP and 150 ng of genomic DNA. Amplified products were electrophoresed on 8% denaturing Polyacrylamid gel. To visualize the PCR product, gels were stained using silver staining (Bassam and Caetano-Anolles, 1993). The stained gels were scanned and genotypes were determined. Observed and effective number of alleles (na and ne), observed and expected heterozygosity (Obs-Het and Exp-Het), average expected theoretical heterozygosity (Ave-Het) from Hardy-Weinberg assumptions, Shannon's information Index (I), mean of heterozygosity and polymorphic information content (PIC) were calculated (Hedrick, 1999 & Beigi Nassiri *et al.*, 2007). 13 polymorphic microsatellite markers (as listed in Table 1) were chosen according to the basis of their location in several chromosomes (FAO., 2004).

Results Table 1 summarises the population statistics. All loci deviated from HWE ($p \leq 0.001$) and they were found to be polymorphic in the population generating 63 alleles across the 13 loci analyzed. CSSM047 and CSSM061 showed the highest number of alleles, but CSSM061 was less polymorphic than CSSM047 according to its Shannon's information index. CSSM033 was the most polymorphic marker ($I=1.60$) in Mazandaran buffalo population. The mean of observed heterozygosity was 0.96. The average of PIC value for all markers was equal to 0.63 according to their allele frequency.

Table 1 Summary of Genetic Variation Statistics for 13 microsatellite loci in Mazandaran buffalo

Locus	na	ne	Obs-Het	Exp-Het	Ave-Het	I
CSSM019	4	3.62	1.0000	0.7296	0.7236	1.33
CSSM029	3	2.20	0.9833	0.5503	0.5457	0.86
CSSM033	6	4.56	0.9866	0.7874	0.7810	1.60
CSSM038	4	3.41	1.0000	0.7134	0.7071	1.29
CSSM041	4	2.89	0.9194	0.6600	0.6547	1.11
CSSM043	5	2.03	0.5818	0.5131	0.5084	0.94
CSSM047	7	4.04	0.9825	0.7592	0.7525	1.58
CSSM061	7	3.51	0.9831	0.7210	0.7149	1.47
CSSM0062	5	4.58	1.0000	0.7878	0.7816	1.56
CSSMR070	5	4.43	1.0000	0.7807	0.7743	1.53
BMC1013	5	3.24	1.0000	0.6969	0.6914	1.32
BRN	4	3.69	1.0000	0.7349	0.7289	1.34
ETH003	4	2.68	1.0000	0.6328	0.6275	1.11
Mean	4.85	3.45	0.9564	0.6975	0.6917	1.31
SE	1.21	0.84	0.1145	0.0873	0.0867	0.24

Conclusion Loci deviated from HWE. The association of loci with some genes of economics importance, migration, and high mutation rate of micro satellite may be the cause. The number of alleles and the mean of heterozygosity shows that the heretozygosity of Mazandaran buffalo population was found to be almost higher than buffalo populations of northern India and Anatolian (Arora *et al.*, 2003; Soysal *et al.*, 2005). It can be concluded that a high degree of genetic diversity exist within the buffalo populations of Mazandaran.

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Investigation on efficiency of individual selection system in 31 and 32 Lines of silkworm and its effects on production traits performance in 31×32 hybrid

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Introduction The main objective of silkworm breeding is the progressive improvement of economic traits to increase the profit of silk producers and other sectors of the sericulture industry. There are approximately 2000 different strains of *Bombyx mori* used in silk production worldwide (Reddy, 1986). Twenty one characters influence silk yield quantitatively or qualitatively (Chateerjee *et al.*, 1990). The use of selection indices for genetic and economic improvement of traits in the shortest time will give the greatest improvement (Harris, 1970). Several researchers have shown the heritability of these characteristics in mulberry silkworm. The general aim of selection in animal breeding is to acquire new generation of animals which, under future economic conditions of the production system, are more efficient than the present generation. The goal of the present study is investigation of individual selection based on cocoon weight in comparison to randomize selection in two lines of mulberry silkworm (31 and 32) and its effects on production traits of their hybrid (31×32).

Materials and methods In the first year, 3P (Grand Grand Parent or pure lines) varieties of two commercial Iranian pure line silkworm (including 31 and 32) were reared under standard conditions. All production traits were recorded individually in each population. Then from each variety, were selected 160 individuals including 80 individuals with superior performance (40 male and 40 female) and 80 individuals with medium performance (equal to the population mean). It was assigned a specified number in order to investigation on performance of these individuals at next generations. Thenceforth silkworm eggs were prepared. These individuals were reared and their performance recorded in next generation. Then for investigation of these groups at next generation they were divided into random and selection index lines. At next generation, were reared 2 groups from random and selection lines with 8 families in each and recorded their parameters. Then for each random and selected group each of two studied variety, were prepared 40 batch silkworm eggs and preserved with 48 hours method. At next generation, these silkworm eggs were hatched and reared and recorded. Then commercial hybrid silkworm eggs were prepared with crossing of 31 (Chinese) and 32 (Japanese) pure line at two random and selected groups. Then these individuals were reared at next generation for comparing of their performance at two random and selected groups. The collected data were subjected to statistical analysis of variance test to find out the low significant difference between the parameters two groups in each hybrid.

Results In Table 1, average of produced cocoon number, best cocoon number, middle cocoon number, low cocoon number, best cocoon percentage and low cocoon percentage are shown for treatments of selection system (S) and Randomized system (R). Significant difference was observed between treatments for traits at %5 levels. It was shown individual recording can be used for performance improvement at silkworm varieties. One of reason for improvement of economic characters refers to positive correlation between them. Thus breeding programs and individual selection for these parameters at lines level can improve total production level and economical gains.

Table 1 Mean comparison of production traits for selection (S) and randomized (R) systems[†]

Traits	Produced cocoon no		Best cocoon no		Middle cocoon no		Low cocoon no		Best cocoon %		Low cocoon %	
	S	R	S	R	S	R	S	R	S	R	S	R
31×32	204.2 ^a	199.2 ^b	150 ^a	146 ^b	44.7	42.2	4.5	7.2	73.1 ^a	71.6 ^b	2.2	3.5
	±3.02	±3.92	±3.12	±4.07	±1.48	±1.18	±0.12	±0.09	±1.78	±1.14	±0.03	±0.05

[†]For each trait, means with the same letter have no significant difference.

Conclusions It is shown that random and selected hybrids have significantly different performance in many parameters and selected hybrids have superior performance ($P < 0.05$). Knowledge regarding sensitivity of economic values to various factors can help silkworm breeders to concentrate on the most important factors in the future market. Furthermore, when important factors change, silkworm breeder will be able to determine to what extent breeding goals have to change. Defining future scenarios for agricultural production and deriving economic values of genetic improvement for these scenarios is a useful tool in developing breeding strategies that are robust to changes in markets. Therefore, it is necessary to study the effect of other constraints of production system on economic values of important traits in silkworm varieties.

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Predicting bovine milk urea concentration for future test-day records in a management perspective

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Introduction Urea is the major contributor to nonprotein nitrogen fraction of milk which represents 5 to 6% of the total nitrogen in milk. Milk urea (MU) nitrogen is closely related to blood urea nitrogen which is derived from at least two sources: the liver detoxification of ammonia diffused from the rumen and the amino acid catabolism in the liver (Depeters and Ferguson, 1992). Thereby, MU concentration could reflect the protein metabolism in the cow and be related to the diet. Several studies showed significant links between MU concentration and nutritional variables (mostly dietary crude protein and energy:protein ratio) or environmental factors (e.g. season or stage of lactation) (Broderick and Clayton, 1997; Schepers and Meijer, 1998; Godden *et al.*, 2000). MU has proved to be an interesting management tool for breeders (Jonker *et al.*, 2001). The aim of our research is to provide feed management tools to Walloon dairy farmers based on the detection of 'abnormal' values. To develop such a tool, MU concentrations need to be predicted for future test days. Given the nature of MU, this presents a special challenge and this study will show first results obtained when testing two different models.

Materials and methods Data used in this study were MU test-day concentrations measured by Mid-Infrared spectrometry and collected from January 1997 to June 2007. Only data from first lactation cows and from days in milk < 365 were used. Milk urea records <10mg/l and >2000mg/l were also deleted. Data set represented 1,749,257 test-day records from 223,969 cows. Data for 17,100 test-day records observed in June 2007 was considered unknown and use to test prediction ability. Pedigree data were extracted from the database of the official Walloon genetic evaluation. Prediction of future MU test-day results was made using two single-trait random regression test-day models. Both models included 4 fixed effects (overall mean, herd, month of test and stage of lactation combined with parity, breed and age at calving) and one random effect (herd test-day) and three random regression effects (herd by year of calving, permanent environment and genetic). Regression curves were modelled using Legendre polynomials of order two. The second model tested also included an autoregressive covariance structure for the herd test-day effect as described by Wade and Quaas (1993). It allowed to model correlations between successive test-day records. Covariances were obtained from 10 random samples using AI-REML (Misztal, 2007). Model fit for the two models were compared by studying distribution of residuals on data prior to June 2007. Accuracy of prediction for MU concentrations in June 2007 were studied by computing prediction errors (PE) for both models.

Results The average MU concentration for the dataset was 288.5 ± 100.9 mg/l. The (co)variance structure showed that the total variance was mainly explained by the residual ($\pm 25\%$ of total variance) and the herd test-day effects ($\pm 50\%$ of total variance). Base on these results, we can speculate that the influence of local events (e.g., diet related) have large implications on the predictability of successive urea records. Table 1 indicated that both models did not fit perfectly the data even if overall fit was equivalent. The average PE was positive with both models, indicating that MU concentrations were systematically underestimated. There was a large range of prediction error standard deviation. However, the second model limited the bias (10.7 instead of 14.3) and improved the accuracy of prediction (0.53 instead of 0.51).

Table 1 Adjustment and accuracy of prediction for the models tested

	Adjustement (1,732,157 records)		Accuracy of prediction (17,100 records)	
	Distribution of residuals	Correlation between observed and predicted MU	Distribution of PE	Correlation between observed and predicted MU
Model 1	0.0 \pm 41.1	0.95	14.3 \pm 87.4	0.51
Model 2	0.0 \pm 41.1	0.95	10.7 \pm 85.9	0.53

Conclusions and perspectives Our research identified some problems for the modelling and the prediction of milk urea concentration. Results demonstrated the interest of the autoregressive covariance structure. Further improvements are needed. Alternative ways to model seasonal trends or to take into account the residuals in a given herd could be necessary.

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Consistency of aggressive feeding behaviour in dairy cows: effect of feedface length, stage of lactation and dominance rank

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Introduction Understanding individual differences in temperament traits enables us to investigate the relationship between those traits with animal production and welfare. One aspect of dairy cow temperament that is particularly important to study is aggression. Stress caused by aggressive interactions can negatively affect behaviour and feed intake (Olofsson, 1999), and cause some cows to alter their feeding times to avoid aggressive interactions (Miller and Wood-Gush, 1991). The objective of this study was to characterise the competitive 'style' of individual cows. The following questions were addressed: 1) are cows consistent in how they express aggression over time at a standard length feedface, 2) does the parity, feedface length, stage of lactation and social dominance rank have an effect on aggressive behaviour?

Materials and methods Ten primiparous and 30 multiparous healthy lactating cows were used (parity = 3.5 ± 2.15 ; mean \pm S.D). Feeding behaviour was recorded for 5 days at a standard (0.6m per cow) and reduced (0.3m per cow) feedface. The standard feedface was recorded for three periods over the animal's lactation at 90 day intervals. The reduced feedface was recorded once. The aggressor and recipient behaviours for each aggressive interaction observed during the 60 minute period following feed arrival were recorded. Aggressor behaviours (contact (C) and non-contact (NC)) and recipient behaviours (non-responsive (NR), active avoidance (AA) and aggressive responsive (AR)) were recorded. A displacement index (DI = no. of active displacements/no. of active displacements + no. being displaced), aggressive index (AI = no. of times aggressor/no. of times recipient + no. of times aggressor) and dominance index (DOI = DI + AI) was calculated for each cow. The DI ranges from 0 to 1 corresponding to always being displaced and successfully displacing others respectively. The AI values range from 0 to 1 indicating always being a recipient and always an aggressor respectively. The DOI takes into account animals that may have a high AI score but a low DI score indicating the rank of an individual depends not only on how often it performs aggressive actions but also on how successful it is. The DOI values ranged from 0 to 2 corresponding to low and high rank respectively. Parity, stage of lactation and dominance rank were factored into the Generalised Linear Mixed Model analysis.

Results DOI was significantly consistent across the three repeats. The DOI negatively correlated with AA ($r_s = -0.79$, $P < 0.001$) and positively with NC aggressor behaviours ($r_s = 0.47$, $P < 0.01$). Higher ranking animals display greater C ($W_2 = 85.76$, $P < 0.001$) and NC behaviours ($W_2 = 16.33$, $P < 0.001$) as aggressors and show less AA ($W_2 = 36.57$, $P < 0.001$) as recipients. Cows in early lactation (<100 DIM) have higher DI ($W_2 = 6.40$, $P < 0.05$), show greater AA ($W_2 = 9.61$, $P < 0.01$) and consequently fewer NR behaviours ($W_2 = 22.18$, $P < 0.001$). C ($W_4 = 14.10$, $P < 0.01$), DI ($W_4 = 12.53$, $P < 0.05$) and AI ($W_4 = 16.61$, $P < 0.01$) increased from first to fourth parity. There was no effect of feedface length on a cow's DI ($U_{40} = 705$, $P = 0.36$), AI ($U_{40} = 723.0$, $P = 0.46$) and DOI ($U_{40} = 703.5$, $P = 0.36$). A statistical summary of standard and reduced feedface length for the remaining behavioural variables are shown in Table 1.

Table 1 Statistical summary of aggressive behaviours between standard and reduced feedface length..

Behaviour	Feedface Length median (Q1-Q3)		U_{40}	Sig	r_s	Sig
	0.6m	0.3m				
					(df=38)	
C	0.36 (0.25-0.46)	0.45 (0.32 - 0.57)	594	*	0.34	*
NC	0.07 (0.04-0.15)	0 (0-0.07)	404	***	0.41	**
AA	0.40 (0.29-0.55)	0.24 (0.2-0.39)	459	***	0.26	NS
NR	0.08 (0.04-0.13)	0.18 (0.09 -0.28)	427	***	-0.06	NS
AR	0.02 (0-0.04)	0 (0-0.05)	615	NS	0.26	NS

U_{df} Mann Whitney Test Statistic, r_s Spearman Rank Correlation Coefficient, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS Non Sig.

Conclusion In this study, cows showed consistent DOI over time and across situations. High ranking cows are more aggressive than mid and low-ranking cows. Early lactation cows are more responsive to aggressors by active avoidance and are more successful in displacing other cows at the feedface. When space allowance decreased, cows avoided aggressors less and aggressive contact behaviours increased. These results highlight the complexity of aggressive style of cows in a competitive situation. It is important to measure individual aggressive behaviour in order to develop suitable temperament scores that can be used in future breeding programmes or as part of welfare assessment schemes.

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Trade-off decisions in dairy cow feeding preferences

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Introduction In recent years there has been an increasing interest in the issue of farm animal welfare. This interest stems from a belief that many modern livestock production systems do not allow animals to perform a natural range of behaviour, leading to a possible decline in welfare. One method of determining the value of natural behaviours is to perform preference tests. In dairy cows, choices relating to various treatments, including feeding, shouting, electric shock, hitting (Pajor *et al.*, 2003) and being milked (Prescott *et al.*, 1998) have been assessed using Y-maze methodology. This is a process that involves training animals to anticipate receiving a treatment if they enter one arm of the Y and an alternative treatment if they enter the other. We used preference tests as a tool to determine how dairy cows perceive aspects of their feeding environment, with specific emphasis on understanding what difficulties low ranking animals face at the feed-face. We hypothesised that subordinate cows would trade-off proximity to a dominant individual at the feed-face with access to food of a high quality.

Method and materials Three groups of 10 lactating Holstein Friesian cows were used in the study ($n = 30$). Half of the cows were multiparous (parity = 3.4 ± 0.5 ; mean \pm SD) and half were primiparous. After morning milking, the cows were taken to a holding pen beside a test pen. The cows remained in this pen with no access to feed until testing sessions were completed. A total mixed ration was fed on return to the home pen. Each animal was moved individually from the holding pen and allowed to walk freely towards the test pen. In the test pen was a Y-shaped maze which consisted of an alley (1.2m long) with two arms (3.65m long) at the top. At the end of each arm was either a black or white feed bin and a sheet of plastic of a corresponding colour on the wall. In the first phase of training, half the cows were trained to associate the black bin and high quality food, and the white bin and low quality food. The other half was trained with the opposite combination. The high quality feed was a concentrate pellet, and the low quality feed was a mix of soya and rolled barley, to represent high and low palatability and desirability respectively. During the training period the social status of each cow was assessed by recording the number of displacements of other cows made during peak feeding. The training phase was 4 consecutive days, and was followed immediately by a testing period of 2 - 4 days. The first phase of testing (Choice Test 1) assessed whether the cows had correctly learned the association between bin colour and food quality by repeated trials in which the high and low quality feeds were presented in alternating arms. Cows progressed to the second test phase if they consistently chose the high quality feed. In Choice Test 2, the subordinate test cow was presented with two bins of high quality feed, one of which had a dominant cow feeding from it. In Choice Test 3, the cow had a choice of high and low quality feed, with the dominant cow present at the high quality bin. In this case, subordinate cows had trade-off access to high quality feed with proximity to a dominant individual. For each test, the number of times each cow chose the arm containing the high or low food was recorded. A Chi-squared test was used to test the significance of the difference between treatments ($p < 0.05$).

Results Cows showed a significant preference for feeding alone rather than next to a dominant when they were offered high quality feed on both sides of the Y-maze (Fig. 1). Figure 2 shows that when asked to trade-off choice between feed quality and proximity to a dominant cow, subordinate cows chose to feed alone on the low-quality food ($P < 0.05$).

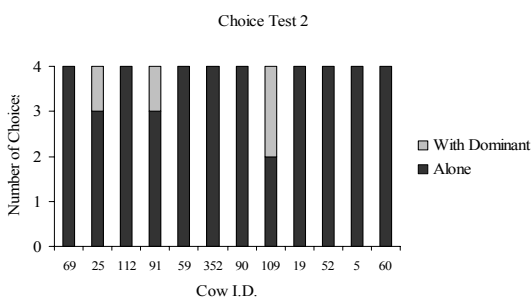


Figure 1 Choice Test 2: subordinate cows given choice of feeding on high quality food alone or next to dominant cow.

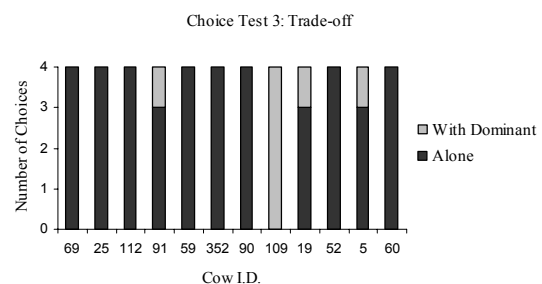


Figure 2 Choice Test 3: subordinate cows given choice to trade-off feed quality with feeding alone or next to dominant cow.

Conclusions When subordinate cows were offered the choice between feeding on high quality feed alone, or next to a dominant, they choose to feed alone, rather than next to a dominant animal. When they were asked to trade-off feed quality with feeding next to a dominant animal, the majority chose to feed alone on a low quality feed. These results imply that the social status within a herd could significantly affect feeding behaviour, especially *in situations* of high competition. This is important to consider for feed management and feed barrier design.

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The effect of organic status, housing type and other management factors on somatic cell counts in dairy cows

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Introduction The measurement of somatic cell counts (SCC) is used in the UK to detect the presence of sub-clinical mastitis in dairy cattle (Mrode & Swanson, 1996). Many studies have investigated the relationship between farm hygiene and management factors on the herd average SCC (from the bulk milk tank) and have shown that factors such as milking parlour hygiene, the use of straw-yard systems, cleanliness of the lying area and mastitis treatment strategies affect SCC (e.g. Barkema *et al.*, 1999; Peeler *et al.*, 2000). The organic status of the farm may also affect SCC. The principles of livestock farming are that animal health should be promoted through good animal care and management, and this is particularly emphasised for organic systems. Organic standards discourage the general prophylactic use of chemically synthesised allopathic veterinary medicines, such as antibiotics. Use of alternative remedies, such as homeopathy, is encouraged for the treatment of disease. Antibiotics can be used in acute cases, such as for clinical mastitis, but the withdrawal period for sale of milk is longer on organic farms than for non-organic farms. There is concern that these regulations may have a negative impact on animal health and welfare, particularly if alternative health treatments are ineffectual. The aim of this study was to determine whether organic status and other farm management practices affect SCC in organic and non-organic farms.

Materials and methods Forty organic and 40 non-organic farms across Great Britain were recruited for this study. Organic and non-organic farms were paired for housing type, and matched for herd size, cow genetic merit for milk production and geographical location as far as possible. The farms used in the study had predominantly Holstein Friesian cows and a herd size of greater than 50 cows. All farms were registered with one of the two major milk-recording companies. Somatic cell counts were extracted from the databases of these companies for all cows on the study farms for the year 2004. During this year, each farmer was interviewed and asked about the farm management routines, milking parlour hygiene practices and the methods used to treat udder disease. In accordance with the standard practice for the assessment of somatic cell count, SCC were log-transformed and only cows with 5 or more test-day records were included in the analysis. Lactation number, milk yield over the lactation, stage of lactation and the season of calving are all factors known to affect somatic cell count, so they were all included in the analysis. Using a univariate model, potential explanatory variables were screened for significance. A sequential stepwise selection process using LMM by REML methods (Genstat 7) was used to decide on the final multivariable model.

Results The mean overall SCC for the 80 farms was 214,000 cells/ml ($\pm 1,600$). The national average for 2004 was 180,000 cells/ml (MDC, 2004). As shown previously, SCC increases with the lactation number of the cow, decreases with increasing lactation yield, varies across the year and across lactation (Mrode and Swanson, 1996). There was no difference between organic and non-organic farms ($P > 0.05$). Many organic farmers stated that they were more likely to treat mastitis in the early stages with an alternative remedy, and to treat with antibiotics if the case became clinical or took too long to clear. Non-organic farmers used antibiotics at the first signs of mastitis. There was no difference between farms where cows were kept in straw-yards or farms using cubicles ($P > 0.05$). The method used to wash the udder before milking also affected SCC ($P = 0.01$), with simple washing or no contact being associated with lower SCC than if dry wipes, dry wipes with washing or wet wipes were used. SCC was higher on farms with small numbers of cows ($P < 0.05$).

Conclusions The results suggest that organic regulations are not compromising the health and welfare of dairy cows with regards to SCC. The use of straw-yards was not associated with higher SCC, as suggested by others (Peeler *et al.*, 2000). The result for udder hygiene seems counter-intuitive, as it suggests that 'less-than-ideal' practice (not using the most sterile udder-cleansing methods) is beneficial for SCC. However, as some farmers may use the wipes on more than one cow, doing little or washing with water may reduce the chances of spreading infection. It appears that the alternative treatment strategies of the organic and non-organic farmers have the same outcomes in terms of SCC.

Acknowledgements This project was sponsored by Defra. NMR and CIS kindly allowed us access to their milk recorder databases. We would like to thank all the farmers who participated in the study, and OMSCo, the Soil Association and the Kingshay Farming Trust for help in recruiting farmers to the study.

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Metabolic profile throughout lactation in primiparous Holstein cows raised in Greece

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Introduction The use of metabolic profiles is widely accepted as an assessment method of the dairy cows' energy status (Whitaker, 2004). Three of the most reliable parameters that can be used are serum concentration of glucose, beta hydroxybutyric acid (BHB) and non-esterified fatty acids (NEFA). There is growing interest in the changes of these parameters during lactation (Wathes *et al.*, 2007). The scope of this study was to record and characterise the fluctuation of serum levels of glucose, BHB and NEFA during a 305-day lactation of primiparous Holstein cows raised under commercial conditions.

Materials and methods The study was conducted in a large commercial dairy farm (900 cows) located in Northern Greece; 359 heifers calving between January 2005 and June 2006 were considered. Animals were fed a total mixed ration. Blood samples were taken weekly from calving to week 13 of lactation and thereafter monthly until the end of lactation. All samples were collected after the morning milking. Blood was centrifuged 30 minutes after sampling and serum was stored at -20°C until analysed. Serum glucose and NEFA concentrations were determined with commercial kits (Glucose liquid fast, Zafiroopoulos diagnostica, Wako NEFA C kit) using a Hitachi spectrophotometer. BHB concentration was determined using an enzymatic kinetic reaction. Data were analysed with linear models including the fixed effects of year-season of calving, age and body condition score at calving, and stage of lactation; cow was also included as a random effect. Stage of lactation was modelled with a fixed regression on 3rd order Legendre Polynomials of week of lactation. This represented the fixed lactation curve of each trait adjusted for all other effects. Individual animal deviations from this curve were modelled with a random regression on the interaction between cow and 4th order Legendre Polynomials of week of lactation.

Results Estimated fixed curves of glucose, BHB and NEFA, by week of lactation, are shown in Figures 1, 2 and 3, respectively.

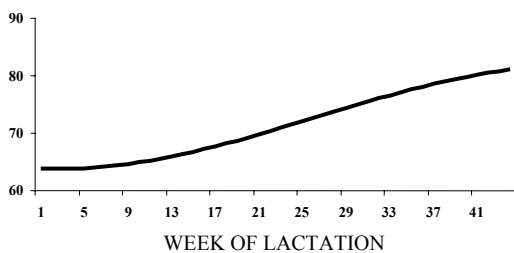


Figure 1 Glucose (mg/dl)

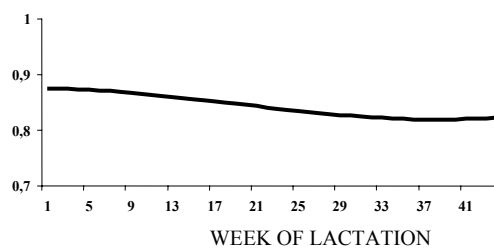


Figure 2 BHB (mmol/l)

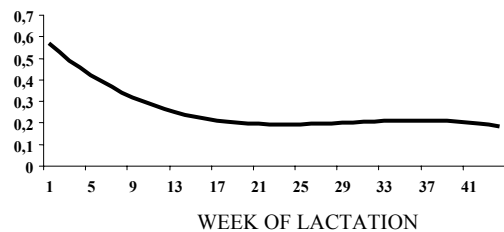


Figure 3 NEFA (mmol/l)

Increasing glucose and decreasing BHB and NEFA during lactation are indicative of changes in the metabolic process and energy balance of the cows. Body condition score at calving had a significant ($P < 0.05$) impact only on NEFA concentration; the average effect over the entire lactation was 0.0326 ± 0.0078 mmol/l per unit of body condition score increase. The proportion of the total variance of the three traits due to the cow effect was estimated. This ratio, which is equivalent to the upper limit of heritability, ranged: a) from 0.15-0.42 for glucose being higher at the beginning and the end of lactation, b) from 0.40-0.50 for BHB during the first two weeks of lactation, being practically zero for the other weeks and c) from 0.11-0.36 for NEFA during the first six weeks of lactation, being practically zero thereafter.

Conclusion The curves obtained effectively reflect the changes in energy balance that dairy cows face during lactation. The sizeable between-cow variation in the studied metabolic traits, especially at the beginning of lactation, is quite interesting as it suggests selection opportunities. Furthermore, the possibility that this variation may explain between-cow differences in fertility and health traits needs further investigation.

Acknowledgements Vivartia ABEE is gratefully acknowledged for providing data and farm resources. Funding was from the General Secretariat for Research and Technology of the Greek Ministry of Development. The first author acknowledges financial support from the Greek State Scholarships Foundation (IKY).

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An investigation into combinations of energy balance and luteal cycle characteristics which influence the day of first heat in dairy cattle

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Introduction Energy balance is often considered to be a major factor in the onset of dairy cow reproductive activity in early lactation. The relationships between various aspects of energy balance (EB) and the day of first heat (DFH) are weak when analysed with the mixed generalised linear model approach (e.g. Butler *et al.*, 1981). This is understandable since DFH may not respond to the additivity of the factors which influence it but may require various states to be reached together before the expression of heat occurs. This study investigates a number of factors which may combine to influence DFH to see which combinations appear to be critical for first heat to occur. The aim of this study was to see if an alternative approach to investigating the link between energy balance and day of first heat would provide a stronger relationship than currently found with the mixed model approach.

Materials and methods The Scottish Agricultural College maintains a fully recorded 200-cow Holstein dairy herd, at its Crichton Royal Farm, which is split between two genetic groups and two production systems. This provides data from cows with a wide range of EB characteristics. The milk from these cows was sampled and analysed for progesterone content (Prog), which was used as an indicator of luteal cycling activity. Farm observations relating to fertility were also used in this study. Daily energy balance was calculated from daily liveweight and smoothed, weekly condition score measurements. Characteristics of the EB curves, such as day of return to positive EB (PEB) and high nadir of EB level (NEB), were used in combination with other aspects of the cows in early lactation (progesterone level, lactation number) to see which combinations of them influenced DFH. Each luteal cycle, as indicated by progesterone levels $>3\text{ng}/\mu\text{l}$, was characterised as being preceded by a heat opportunity and heat opportunities up to the first farm observed heat were analysed. A series of chi-squared analyses were undertaken using various combinations of PEB, NEB, progesterone level in the preceding cycle (Prog) and cycle number (CN) with the occurrence of first heat as the measured variable (0= no heat; 1 = heat).

Results Data from 456 heat opportunities expressed in 188 lactations from cows which had a healthy lactation (i.e. no recorded incidence of mastitis, metritis, lameness, cystic ovaries) were analysed. Whether the cow was in PEB at the start of the previous luteal cycle (or not) influenced the incidence of first heat occurring ($P>0.001$) but 138 heat opportunities occurring after PEB did not result in a heat and 58 heats were expressed before PEB had been reached. Combining Prog with PEB and reanalysing the heat expression data resulted in a higher than expected number of heat opportunities when both factors occurred together ($P>0.001$) and lower than expected number when neither occurred together (Table 1). However there were still a number of heats expressed when both PEB was not reached and Prog was low (31). Further analyses combining PEB, Prog, NEB and CN together indicated that few 1st cycles were preceded by observed first heats and that PEB combined with Prog and/or NEB resulted in more than expected DFH occurring ($P>0.001$). Also the combination of no PEB and NEB resulted in more than expected numbers of DFH.

Table 1 The occurrence of day of first heat at a heat opportunity with different combinations of positive energy balance (PEB) and progesterone level in previous heat (Prog).

		Day of first heat		Total
		0	1	
No PEB Low Prog	Count	119	31	150
	Expected Count	96.1	53.9	150.0
No PEB High Prog	Count	35	27	62
	Expected Count	39.7	22.3	62.0
PEB Low Prog	Count	70	34	104
	Expected Count	66.6	37.4	104.0
PEB High Prog	Count	68	72	140
	Expected Count	89.6	50.4	140.0

Conclusions Early lactation fertility appears to be influenced by both the return to positive energy balance and the level of energy balance nadir but other factors, such as the level of progesterone in the preceding cycle and whether a cycle is the first in the lactation also critically affects the expression of heat.

Acknowledgements This work was funded by the DEFRA LINK scheme with the co-operation of industrial sponsors; National Milk Records, Cattle Information Services, Genus, Cogent, Holstein UK, BOCM Pauls, Dartington Cattle Breeding Trust and RSPCA. SAC receives financial support from the Scottish Executive Environment & Rural Affairs Department.

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Observations on the time course of calving events in unassisted multiparous spring calving suckler cows housed in a straw bedded yard

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Introduction As financial pressures on UK beef farms increase, each beef industry stockman will be expected to look after more animals. Consequently, labour availability around calving will be reduced in future years. In order to enable limited human assistance to be targeted towards those cows and calves that need it, a sound understanding of the time course of the natural calving process is needed. The objective of this study was to detail the time dynamics of the major externally visible calving events in unassisted spring calving suckler cows housed in a straw bedded yard, typical of UK practice.

Materials and methods The entire duration of the calving process in 23 multiparous spring calving suckler cows was recorded using a multiplex digital camera system and the continuous images were stored for subsequent processing by a human operator. Externally visible calving events were recorded using behaviour recording software (Observer 5). The calving events were defined as: 1st appearance of the waterbag, waterbag rupture, 1st sight of the calf, calf head out, calf shoulders out, calf hips out, fully calved, 1st time calf seen standing, 1st time calf seen standing at the udder and 1st time calf seen sucking. The time durations between each of these events were also recorded. Suckler cow breed types were either Aberdeen Angus cross Limousin (AAx) or Limousin cross Aberdeen Angus (LIMx) according to a two-breed rotational cross breeding programme within the SAC suckler herd. All AAx cows were always mated to a pure LIM sire whilst all LIMx cows were always mated to a pure AA sire to maintain the two-way rotational crossbred suckler cow genotype. Cow liveweights (LW) and body condition scores (BCS) were determined approximately one month before the predicted start of the calving period and calf birth weights (BW) were recorded at birth. A total of 11 and 12 data sets were available for the AAx and LIMx cow genotypes respectively with 9 male and 14 female calves being born during the study. All data was statistically analysed using the residual maximum likelihood procedure (REML) in Genstat 5 according to a 2 x 2 (Cow/calf genotype x Calf-sex) continuous factorial design.

Results Only the between calf-sex effects are given in Table 1 since cow/calf genotype was not statistically significant. Due to very small time durations in the immediate pre-calving and other periods, it was necessary to group the above events into the longer main events described in Table 1. The range in individual time durations of the main calving events are also shown in Figure 1. The time taken for male calves to stand post calving and to suck once they had stood was significantly longer ($P < 0.01$) than for female calves. The total time duration of the entire calving process from 1st sight of the waterbag to calves sucking was also significantly longer ($P < 0.05$) for male compared to female calves.

Table 1 Animal weight, age and condition parameters along with time durations (minutes) for the main calving events in unassisted spring calving suckler cows according to the sex of the calf

	Male	Female	sed	Sig		Male	Female	sed	Sig
Cow LW (kg)	689	723	29.6		Waterbag to calf seen	77.3	43.9	23.63	
Cow BCS (1-5)	2.58	2.61	0.105		Calf seen to fully calved	15.0	11.2	4.77	
Cow age (parities)	4.0	5.3	0.84		Calving to calf standing	58.3	31.4	7.88	**
Calf BW (kg)	44.7	43.9	1.67		Standing to 1 st suck	44.6	15.4	10.19	**
					Total time duration	195.3	102.0	35.26	*

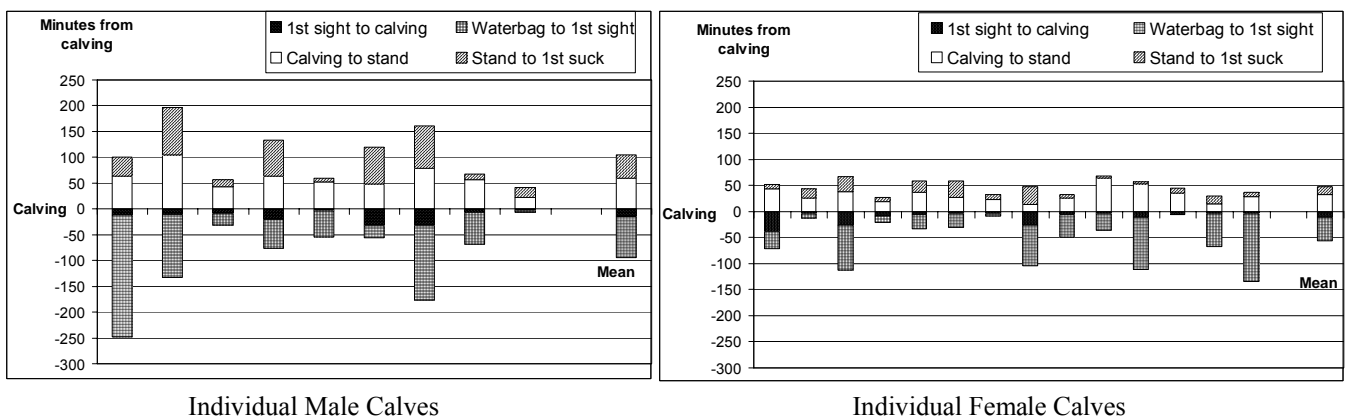


Figure 1 The time course of the major, externally visible calving events in unassisted spring calving suckler cows.

Conclusions Even where cows apparently required no assistance at calving and despite little difference in calf BW between sexes, the duration of the calving process was longer for male calves compared to females and they also took longer to stand and suck than female calves. Post calving, scarce labour resources may most usefully be targeted towards male calves.

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Risk factors for piglet mortality in outdoor farrowing systems

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Introduction Outdoor farrowing system account for 27% of breeding sows in England, and it is therefore important to understand the factors affecting their production efficiency. Piglet mortality is a significant economic and welfare issue, with farrowing and preweaning losses in commercial outdoor herds currently averaging 19% of all pigs born. Since sophisticated environmental and human interventions at the time of farrowing are infeasible in these systems, risk factors may differ in importance from those seen in indoor systems. A better understanding of the risk factors for different causes of mortality will help to design strategies to minimise losses.

Methodology As part of a large genetic research programme (Roehe *et al.*, 2007), data were collected on 17191 individually identified piglets from sows farrowing over 3 parities in individual outdoor paddocks on Scottish farms over a 15 month period. For each piglet, litter information, gender, birthweight, fostering history and survival were recorded. All piglets which died before weaning were subject to post mortem investigation to determine cause of death. The data were used to investigate the influence of different factors on prenatal mortality (born dead) and postnatal mortality (dying during the suckling period), by fitting a generalised linear model using the logit link function of the Genmod procedure of SAS. A risk factor analysis was carried out, fitting farrowing month and parity (a single confounded variable), gender and gestation length as fixed class variables. Litter size, litter birthweight variation and piglet birthweight were fitted as covariables, with birthweight fitted by a linear-quadratic-cubic regression (higher order regressions were non significant for other factors).

Results From a mean total litter size of 13.6 ± 2.87 (SD) piglets, overall survival at birth was 97.3% and survival during the nursing period was 90.15%. The major cause of postnatal mortality was crushing, accounting for 73-84% of all identified causes of death in different parities. Low vitality, starvation and scour each accounted for less than 10% of losses per parity. Factors affecting prenatal and postnatal mortality are shown in Table 1. The pattern of mortality risk with month/parity was inconsistent. Male pigs were at 1.16 greater risk of stillbirth (ns) and 1.19 risk of subsequent mortality ($P < 0.01$) than females. Risk of both prenatal and postnatal mortality reduced progressively for each 0.1 increment in birthweight between 0.5 and 2.3 kg (Figure 1), and increased progressively for each 0.05 increment in within-litter standard deviation between 0.10 and 0.65. Both parameters increased with sow parity.

Table 1 Factors affecting piglet mortality

Factor	DF	Chi-sq	P-value
Prenatal mortality:			
Month/parity	15	34.02	0.0034
Gender	1	2.45	0.1175
Gestation length	8	23.96	0.0023
Birthweight	1	20.26	<0.0001
(Birthweight) ²	1	11.97	0.0005
(Birthweight) ³	1	10.61	0.0011
Litter bwt variation	1	11.95	0.0005
Total born	1	1.15	0.2835
Postnatal mortality:			
Month/parity	15	97.52	<0.0001
Gender	1	10.3	0.0013
Gestation length	8	18.29	0.0192
Fostering	1	31.25	<0.0001
Birthweight	1	49.72	<0.0001
(Birthweight) ²	1	19.39	<0.0001
(Birthweight) ³	1	11.57	0.0007
Litter bwt variation	1	18.48	<0.0001
Total born	1	3.44	0.064

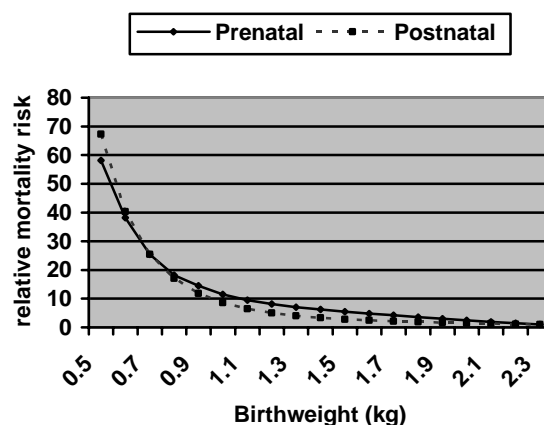


Figure 1 The relative risk of mortality in relation to birthweight (with 2.3 kg as reference weight)

Conclusions: Birthweight and birthweight variability exerted the predominant influence on both prenatal and postnatal mortality under outdoor conditions. Genetic approaches to optimise these traits therefore offer an important opportunity to improve pig welfare and enterprise profitability.

Acknowledgements Financial support of Defra and SEERAD for project LS3103 (“Genomum”) and the substantial support of our partners PIC, JSR, GCP and SSPCA are gratefully acknowledged.

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Welfare indicators for dairy cows in the Northwest of Portugal

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Introduction In Portugal there is an increasing interest in animal welfare studies directed mainly to the improvement of the housing systems. It is recognised that housing is an important factor that affect the behaviour, the production and the health of dairy cattle (Bowell *et al.*, 2003). However, in Portugal few data on the welfare of dairy cattle are available. Therefore this study aimed to determine some housing, health and other welfare indicators in dairy farms in Northwest of Portugal.

Materials and methods Fifty dairy farms in Northwest Portugal were selected at random and a questionnaire was posted to evaluate the welfare of the dairy cows in that region. The selected farms had a minimum of 20 and a maximum of 250 cows. All farms had a cubicle housing systems, which is the most common system in commercial dairy farms in this region. Three groups were established according to the number of cows: G1 (20 to 45 cows), G2 (46 to 90 cows) and G3 (91 to 250 cows). Data were collected during spring of 2005. A report proposed by Haskell *et al.*, (2005) was followed for the evaluation of housing, health and other welfare indicators. Differences between groups were tested using one-way ANOVA. The comparison of averages was carried through by the test of Fisher-PLSD.

Results The characterization of the dairy farms and its welfare indicators for the 3 groups are presented in Table 1. Overall there are no significant differences between groups. However, some housing welfare indicators must be pointed out. This is the case of the number of cubicles which was lower than the number of cows for all groups. This is an important housing welfare indicator since it may be related with the aggression behaviour and with a reduction of the resting time of the cows. The length of feed face area per cow was lower ($P < 0.05$) for the G3 group. Yet for all groups this housing welfare indicator is above the recommendations. The health welfare indicators show that severe hock lesions were more frequent in G3 as indicated by the larger ($P < 0.05$) diameter of the lesions. For all groups the body condition score was close to values indicated for a good productive and reproductive management of the cows.

Table 1 Farm characterization and welfare indicators in dairy farms in Northwest of Portugal.

Characteristics and welfare indicators		G1 (n= 16)	G2 (n= 19)	G3 (n= 15)
General characteristics	Number of cows	35.7±5.1	66.0±13.2	120.5±39.1
	Daily milk yield (kg/day)	26.5±2.2 ^a	28.9±3.1 ^a	28.0±2.5 ^a
Housing welfare indicators	Number of cubicles	31.8±7.3	65.4±19.3	107.2±42.1
	Cubicles minus cow number	-3.9±6.1 ^a	-0.6±14.5 ^a	-13.3±28.8 ^a
	Cubicles minus cow number (%)	-11.0±16.8 ^a	-0.8±23.4 ^a	-10.2±24.9 ^a
	Cubicle width (m)	1.13±0.05 ^a	1.16±0.05 ^a	1.15±0.05 ^a
	Cubicle length (m)	2.20±0.08 ^a	2.28±0.09 ^b	2.28±0.09 ^b
	Cubicle floor type (%)			
	Concrete	12.5	0.0	6.7
	Mattress	6.3	21.1	20.0
	Mat	12.5	15.8	40.0
	Sawdust	68.7	63.1	33.3
Length of feed face area per cow (m)	0.84±0.21 ^a	0.80±0.24 ^a	0.76±0.24 ^b	
Health welfare indicators	Somatic cells (x 1000)	202.4 ±34.9 ^a	220.9± 73.7 ^a	237.8 ±106.3 ^a
	Linear score	3.84± 0.23 ^a	3.79± 0.54 ^a	3.87± 0.64 ^a
	Hock lesions (%)			
	No lesions	75.0	31.6	38.8
	Calloused areas	18.8	47.3	21.0
	Scratches and swelling	6.2	21.1	40.2
	Lesion diameter (cm)	3.8 ±1.7 ^a	4.5± 2.4 ^a	10.8± 4.6 ^b
Other welfare indicators	Body condition score	3.1 ±0.5 ^a	2.7 ±0.5 ^a	3.2 ±0.5 ^a
	Cow cleanliness (score 0 to 5)	3.3± 0.7 ^a	2.1± 0.9 ^b	3.0± 1.2 ^a

Within a row means with a different superscript letter differ significantly ($P < 0.05$).

Conclusions Despite the low number of farms used in this study, we may conclude that the number of cows has no effect on the welfare indicators studied and that, in general, the welfare of dairy cows is good. The number of cubicles lower than the number of cows in all groups and a higher frequency of severe hock lesions in larger farms were the main welfare problems detected in dairy farms in Northwest of Portugal.

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The effect of altering the floor surface, on the intake and behaviour of housed dairy cows

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Introduction Housed dairy cows spend approximately 4–5 hours per day eating, and normally stand on a concrete surface during this time. However, concrete surfaces are known to contribute to hoof lesions, and subsequent lameness problems. It has been suggested that improving cow comfort at the feeding area, for example, by providing a more ‘comfortable’ standing surface, may promote total dry matter (DM) intake, and improve hoof health and cow welfare. This study was designed to examine the effect on food intake and cow behaviour of placing matting along the standing area behind a feed barrier.

Materials and methods Eighteen late lactation (mean, 398 days in milk) Holstein-Friesian dairy cows (mean milk yield, 14.3 kg) were used in a two-treatment (9 cows per treatment), four period (period length, 10 days), change-over design experiment. Cows were divided into two groups, each of nine cows, with groups balanced for lactation number, days calved, milk yield, live weight and condition score. The two groups were housed separately, side by side, in cubicle accommodation. The floor area of each pen comprised an un-grooved concrete apron (1.5 m wide) behind the feed barrier, and a slatted area (2.1 m wide), each of which ran the full length of the pen (13.6 m). Each pen was equipped with a single row of nine cubicles. The feed barrier comprised a 0.55 m high stub wall, and a 1.20 m high top rail. The length of feed space within each pen was 5.3 m (0.59 m/cow). A 28mm thick compression moulded mat made from expanded polymers (Pad-Mat, UVr D27 4L, Pemarsa S.A., Alicante, Spain), measuring 7.2 m x 2.3 m (density, 0.27) was attached to the floor behind the feed barrier in one pen. The two groups of cows rotated between pens, so that each group was on each treatment (Mat or Concrete) twice. Grass silage was offered *ad-libitum* once daily, at approximately 10:00 h, with feed being pushed up on four occasions each day. Uneaten food was removed at 09:00 h the following day. Cows were milked twice daily (between 06:00–08:00 h and 14:00–16:00 h, and were offered 1.5 kg concentrate in the milking parlour at each milking. Feed intakes were measured during days 6–10 of each experimental period. On day nine of each period, a group scan was undertaken every 15 minutes, from 10:15 to 00:00 h (excluding milking time), and the location of animals within the pen noted. Animals were identified as either being at the ‘feeding area’ (defined as having at least two feet on the mat, or equivalent area of the concrete apron), in the ‘alleyway’ (defined as standing on the concrete apron or slatted area, but excluding the ‘feeding area’), or on the ‘cubicles’ (defined as being either completely or partly on the cubicles). In addition, at each group scan, the number of animals involved in a range of activities (eating, standing at the feeding area, standing/walking in alleyway, drinking, standing in cubicle, and lying in cubicle) were recorded. Mean group feed intake data for the final five days of each experimental period, and mean behavioural data for the 12-hour observation period, were analysed by ANOVA, based on four replicates per treatment.

Results Total DM intakes were 15.4 and 15.8 kg/day (SEM, 0.22) with the Concrete and Mat treatments respectively ($P>0.05$). The number of animals observed at different locations within each pen, and the number of animals involved in a range of activities (over a 12-h observation period) are presented in Table 1. Treatment had no significant effect on any of the parameters measured ($P>0.05$).

Table 1 The effect of treatment on the number of animals observed in various locations within each pen, and on the number of animals involved in a range of behaviours, over a 12 h observation period

	Concrete	Mat	SEM	Sig
Location within pen				
Feeding area	3.0	3.3	0.16	NS
Alleyway	1.0	0.7	0.23	NS
Cubicles	5.0	5.0	0.08	NS
Activity				
Eating	2.8	2.9	0.15	NS
Standing at feeding area	0.1	0.4	0.11	NS
Standing/walking in alleyway	0.7	0.4	0.22	NS
Drinking	0.4	0.3	0.04	NS
Standing in cubicle	0.8	1.0	0.57	NS
Lying in cubicle	4.2	4.0	0.23	NS

Conclusions Placing a compression moulded mat behind a feed barrier had no significant effect on either intake or cow behaviour, compared to cows standing on concrete.

Acknowledgements This study was jointly funded by DARDNI and AgriSearch. L.Stroebe acknowledges support from CAH Dronten, Netherlands.

Breed effect on lamb activity and vocalization in a man-animal interaction test

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Introduction The interaction between man-animal has a great impact on farm animal welfare and it constitutes an area of dynamic research. Typically the goal this kind of studies is to reach good management practices with lower costs and providing a better welfare to the animals. To evaluate the interaction man-animal many behavioural tests have been developed and applied to different animal species (Pajor *et al.*, 2003; Lansade *et al.*, 2004; Tallet *et al.*, 2006). These tests are used to evaluate relationship between farm animals and their stockperson and how it affects the animal behaviour. The objective of this study is to compare the lamb behaviours of activity and vocalization of two breeds - Île-de-France (IF) and Churra da Terra Quente (CTQ) in a man-animal standard test.

Material and methods The experimental group consisted of 12 lambs of each breed (CTQ and IF) with 88 ± 11 days of age, kept under the same feeding and management conditions. The studied lambs and their mothers were lodged in two equal pens, one for each breed, contiguous to the test area. For the test it was followed a procedure similar of that described by Tallet *et al.* (2006). Briefly, this test consists of placing individually each lamb in an unfamiliar environment, which is the test area. This test area consists in a 5.5 x 2 m pen surrounded by a 1.8 m high blind wooden border, which did not allow the lambs to have visual contact with the other animals and with stockperson. This test pen was divided in 5 areas (1 x 2 m) by white lines painted on the floor and in the wooden borders and one area of 0.5 x 2 m, in which the stockperson seated (Figure 1). A video camera (SONY Handycam 2006i) was placed in a high position in order to catch all the test area. The test was divided in 3 different situations (S1, S2 and S3) of 2 minutes each: S1 - the animal was alone in the test pen; S2 - the stockperson entered the pen and sat at the end of the test pen and S3 - the stockperson left the pen and the animal was again alone. Audio and video records were used to evaluate the lamb behaviour. These records were digitised and analysed in a computer and the vocalization and activity behaviours were evaluated. The activity behaviour was determined by image analysis using Image J software as the total lamb movement inside the test pen. The vocalization behaviour was measured by audio analysis during the activity behaviour determination. Statistical analyses were performed using the JMP-SAS (Version 5.1; SAS Institute Inc. Cary, NC, USA). The data were analysed by a factorial, being studied the breed and situation effect. Mean comparison was carried by the test of Fisher-PLSD.

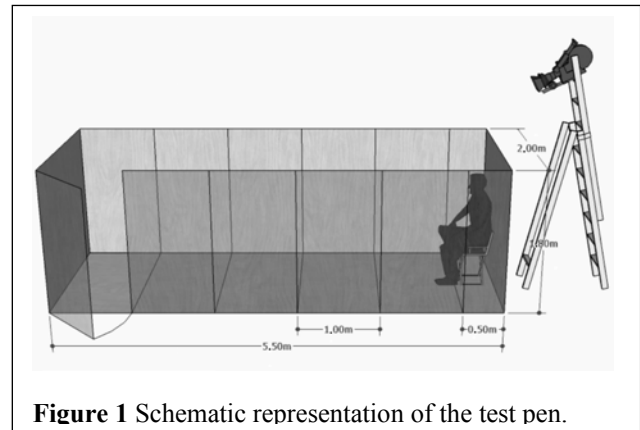


Figure 1 Schematic representation of the test pen.

Results The Figure 2 shows the variation of the activity at S1, S2 and S3 for IF and CTQ breeds. The results show that IF lambs present more activity in all the situations than CTQ lambs and the S1 and S2 are always the situations where lambs show respectively high and less activity ($P < 0.05$), which reflects the presence of the stockperson. Similarly, vocalization behaviour was reduced with the presence of stockperson, which is the S2 situation, and was lower for CTQ lambs.

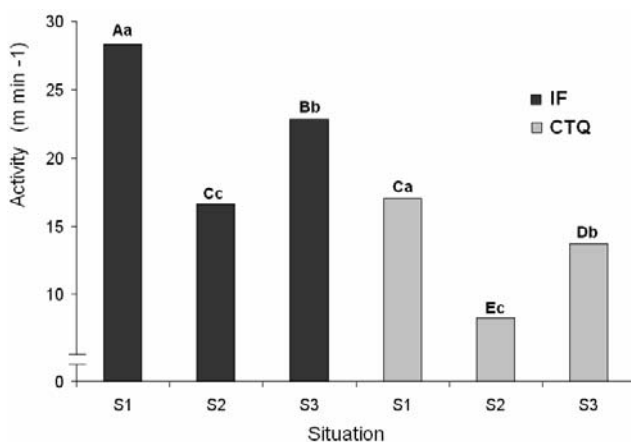


Figure 2 Behaviour activity for the test situations S1, S2 and S3 for IF and CTQ breeds. For breeds, bars with different capital letter are significantly different ($P < 0.05$). For situations S1, S2 and S3 and for each breed bars with different small letter are significantly different ($P < 0.05$).

Conclusions We may conclude that IF and CTQ lambs show different behaviour. The CTQ lambs exhibit lower activity and vocalize less, suggesting a higher ability to be in balance face the test. Therefore, CTQ lambs are potentially easier to handle and the management with this breed will be simplified. For both breeds, the stockperson presence always results in a reduction of the vocalization and activity behaviours.

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Horse gender and fall rate for hurdlers during 2004/05 National Hunt season

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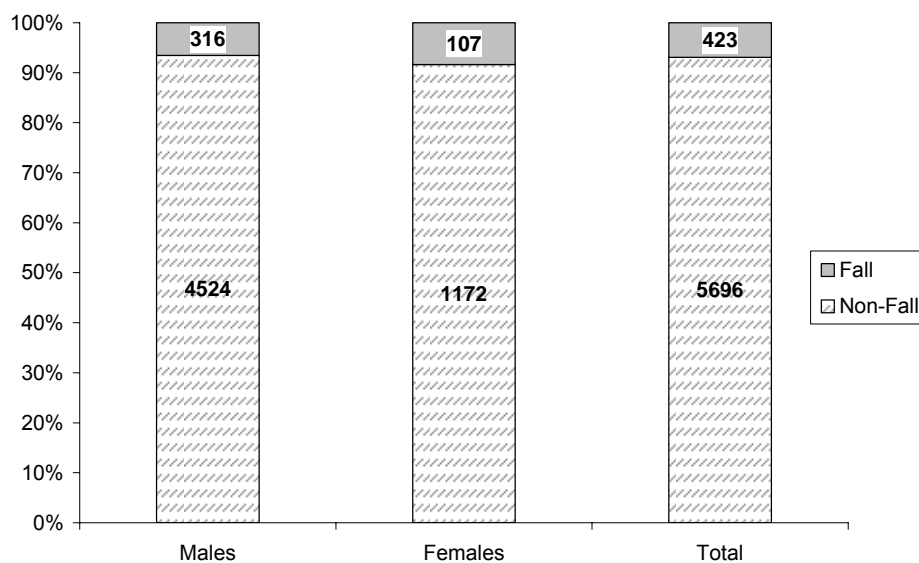
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Introduction Equine welfare is a major concern within the racing industry. A large proportion of fatalities (50-60%) are the result of falls at fences (Pinchbeck, *et al.*, 2004). Various authors have reported factors affecting fall rates both within steeplechase and hurdle races these include; race length, race speed, ground conditions (the going), the horses Timeform rating, previous history, race progression and whip use (Pinchbeck, *et al.*, 2003; Pinchbeck, *et al.*, 2004). At present there have been few studies carried out investigating the effects of gender on the risk of falling in hurdle racing. This study undertook an investigation of fall rates for hurdlers running during the 2004/05 National Hunt season, discriminating via gender.

Materials and method Data was collated from the Timeform (2005) Chasers & Hurdlers 2004/05 form book, for every horse that started in a hurdle race. Cross-tabulation of horse gender and race outcome (faller – non-faller) was performed. Number and percentage fallers were returned with regard to each respective group and in relation to the whole population. The chi square test was used to test for association between gender and race outcome. No information relating to the number of horses ‘pulling-up’ during a race was available within the presented data set.

Results Figure 1 illustrates the numerical breakdown of race outcome discriminated by gender. Chi squared analysis identified that there was a significant association between gender and race outcome ($\chi^2 = 5.305$, $df = 1$, $p < 0.05$). Within group cross-tabulation between gender and race outcome revealed that females had the highest percent of falls at 8.3% of total female runners with male falls at 6.5% of total male runners. A cross population analysis showed males had the highest percentage of fallers 5.1%, females 1.7%. In total 423 horses fell hurdling during 2004/05, 6.9% of the population.

Figure 1 Numerical breakdown of race outcome described by gender and total



Conclusion and discussion The faller rate was higher than that previously reported by Pinchbeck *et al* (2003) of 4.5%. A significant association was shown between gender and race outcome. However the small numbers of stallions within the data set make any interpretation of this discrete group difficult. Quantifying why females had a higher fall rate is difficult and certainly worthy of further study and consideration. Physiological differences in muscle fibre proportions between sexes would suggest that females have less power and stamina with lower proportions of type IIA muscle fibres; therefore fatigue more quickly than stallions (Roneus, *et al.* 1991). However no such differences have as yet been reported between females and geldings (Roneus, *et al.* 1991). Additional consideration needs to be given to both rider and trainer approach to males and females. The much higher percentage of males in training (78.8%) may indicate a confounding effect. However quantifying such effects may prove extremely complex and challenging.

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The effect of GnRH injection and post insemination plasma progesterone concentration on delayed ovulation and conception rate in Iranian Holstein cows

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Introduction Reduced fertility has been had significant impact on economic losses in dairy herds. Ovarian functional disorders are reported as an important cause of infertility in dairy cattle. Among which delayed ovulation affects about 10-15 percent of high yielding cows (Ahmad *et al.* 1996). Progesterone profiles at artificial insemination (AI) and on day 5 post insemination are related to conception rate. A need for adequate concentration of progesterone in early pregnancy is well established and an insufficiency of progesterone has been implicated as a cause for abnormal development of embryos and early embryonic death. Administration of GnRH during the early postpartum period has increased early ovulation, but the effect on the interval from calving to conception has been variable (Britt *et al.*, 1974; Archbald *et al.*, 1990). This experiment was carried out to determine the effects of GnRH injection at the time of insemination on ovulation rate, conception rate and plasma progesterone concentration on day 5 post insemination.

Materials and methods In a factorial design, 80 cows were randomly assigned into two experimental groups: 1) treatment group (40 head) which received 15µg of GnRH analogue (Gonadorelin, Aboureihan Drug Manufacturing Co., Iran) and 2) control group (40 head) without any hormonal treatment. Half of each group (20 head) was palpated regularly per rectum to ascertain the occurrence of ovulation 24 h after insemination. The oestrus was detected by two experienced workers and cows were inseminated by three technicians. They were milked three times daily and were fed a total mixed ration. Blood samples were drawn on the day of insemination and on day 5 post AI. Blood samples were chilled on ice after collection, held at 4°C for 1 hour until serum was obtained by centrifugation, and then stored frozen (-20°C) until concentration of progesterone were measured by RIA. Progesterone concentration was analysed by means of the General Linear Model procedure of the Statistical Analysis System (SAS 8.2). Differences between the two experimental groups were analysed by one way ANOVA. Ovulation rate and conception rate were analysed by means of Binary Models (Logit Regression) of the SPSS 13.

Results Results of this study indicated that the injection of GnRH increased the ovulation rate insignificantly (65% vs 47.5%, $P>0.05$) and increased conception rate significantly (55% vs 25%, $P<0.05$). The apparent increase in plasma progesterone concentration on day 5 post AI, as a result of GnRH administration was not significant (6.033 vs 5.27 ng/ml, $P>0.05$). Rectal palpation had a negative and significant impact on day 5 progesterone concentration (3.761 vs 6.289 ng/ml) and conception rate (31.5% vs 48.75%, $P<0.05$).

Table 1 Conception rate, ovulation rate and plasma mean progesterone concentration on day 5 post AI in treatment and control groups (means \pm SE).

Item	GP	GO	OP	OO
Conception rate, %	50 \pm 4.5	60 \pm 5.7	15 \pm 6.5	35 \pm 3.1
Ovulation rate, %	60 \pm 6.1	65 \pm 6.7	25 \pm 5.4	40 \pm 6.3
Progesterone concentration, ng/ml	6.1 \pm 0.7	6.7 \pm 0.4	3.8 \pm 0.5	4.01 \pm 0.1

GP:GnRH injection at AI and rectal palpation
GO:GnRH injection at AI with no rectal palpation
OP: with no GnRH injection and rectal palpation
OO: with no GnRH injection and no rectal palpation

Conclusion Gonadotropin-releasing hormone (GnRH) given at insemination increased ovulation rate and progesterone in serum and appeared to increase conception rates by increasing embryonic survival during the period of placentation in cows. Improvement of conception following GnRH treatment at AI either can be attributed to the prevention of ovulation failure or to reduced variation in the interval to ovulation. In general, results of this study indicate that GnRH injection at the time of artificial insemination improves ovulation and pregnancy rates.

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Effect of prostaglandin F_{2α} on reproductive performance in Iranian low libido Holstein bulls

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Introduction The widespread use of dairy bull semen in artificial insemination requires that semen production be as efficient as possible. Investigators have shown that low libido is one of the most common problems encountered with bulls and one that frequently leads to culling. In commercial situations, treatment with PGF_{2α} has been used to expedite mounting behavior, as well as restore libido in boars displaying decreased sex drive (Szurop *et al*, 1985). When administered prior to ejaculation, PGF_{2α} markedly increased the number of spermatozoa in first ejaculates from bulls and rabbits (Hafs *et al*, 1974). The objective of this study was to determine whether injections of PGF_{2α} in low libido Iranian Holstein bulls can improve semen characteristics and libido.

Materials and methods Prior to the main experiment, a pre experiment was conducted to select of low libido bulls. In the pre-experiment, 92 bulls were stimulated with two teasers and observed 2 days a wk for 15 min for 3 wks, and their reaction times were recorded. We selected 10 bulls which had longer reaction time as low libido bulls. Selected bulls were assigned to two group (n=5) with similar libido score (Estienne *et al*, 2004). Bulls in the first group were treated with saline (2 mL) and served as control group, whereas bulls in the second group (treated group) received an intramuscular injection of 250 µg Cloprostenol (PGF_{2α} analogue, Nasr LTD, Iran) 30 min prior to collection of the ejaculates on each semen collection day (2 days per wk for 2 months). Two ejaculates from each bull were collected on Saturdays and Tuesdays. Semen was collected in an artificial vagina by experienced semen collector. In the first and last weeks of the experiment when semen was not collected, 3 blood samples were collected at 20 min interval for 1 hour after Cloprostenol or saline injection to determine the effect of treatment on plasma testosterone concentration. Libido was assessed based on reaction time and duration of ejaculation (Estienne *et al*, 2004). Reaction time was defined as the interval from entering the collection room until start of first mounting. Ejaculation time was recorded according to how long ejaculation took place after entering the collection room. Bulls operated on freely and handler had no role. Semen volume, sperm concentration, the percentage of morphologically normal sperm cells, percentage of live sperm cells, motile sperm cells, and post-thaw motile sperm cells were evaluated as semen characteristics. Plasma testosterone was measured by RIA procedure. Semen characteristics, libido and plasma testosterone concentration were analyzed utilizing the procedure MIXED of SAS.

Results The reaction time in the control group (78 ± 3.0 sec) was significantly (P<0.05) greater than that in the treated group (51±2.1 sec). Duration of ejaculation was significantly (P<0.05) increased in treated group (1.5 ± 0.04 sec) compared with control group (1.1± 0.02sec). The treated group had a shorter reaction time and a greater ejaculation time than those in control group (P<0.05). Therefore, the treatment had a positive effect on bull's reaction and ejaculation time. Data collected for semen characteristics over the course of experiment are shown in table 1. There were significant (P<0.05) effects of treatment on semen volume and sperm concentration. Other semen characteristics in treated and control group were not affected by treatment (P<0.05). Plasma testosterone concentrations in treated group (4.1 ± 0.6 ng/mL) were significantly (P<0.05) higher than control group (2.3±0.3 ng/mL).

Table 1 Means (±SE) of semen characteristics over the course of experiment in control and treatment group.

Item	PGF _{2α}	SE	Control	SE	P-value
No. of bulls	5	-	5	-	-
Semen Volume, mL	6.83	0.24	5.72	0.22	0.03
Sperm concentration, million/mL	1146	48	969.06	47	0.02
Motile sperm cells, %	53.92	1.62	51.87	1.57	0.23
Post-thaw Motility, %	36.07	0.75	35.54	0.70	0.42
Morphologically Normal sperms, %	85.46	0.28	85.79	0.25	0.36
Live sperms, %	62.45	1.01	62.92	0.94	0.66

Conclusion The results of this experiment show that injection of Cloprostenol at dosage and frequency used in this study increased libido, semen volume, sperm concentration and plasma testosterone concentration but did not have any effect on motile sperm cells, morphologically normal sperms and live sperms in Iranian low libido Holstein bulls.

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Evaluation of some blood biochemical factors in postpartum anoestrous in dairy cows

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Introduction Over the past decades there has been a substantial decline in the reproductive performance of high-producing dairy cows. The negative energy balance (NEB) early post partum and intake of a high-protein and high-energy diet, are known to cause hormonal and biochemical changes in these cows (Lopez –Gatius *et al.*, 2001). Hence, metabolic and endocrine changes in early lactation allow enhanced mobilization of depot fat and skeletal muscle break down and favour partitioning of absorbed nutrients to the mammary gland to provide sufficient substrate for milk synthesis (Butler, 2002). Anoestrus, a common state during the post partum period in high-producing cows, has major economic implication since it increases the length of the calving interval. Beta hydroxybutric acid (BHBA) is caused by a negative energy balance, and typically is produced within 2 months after calving (Butler, 2002; Noordhuizen, 2003). Anoestrus is a failure to exhibit oestrus behaviour in cyclic cows that classified into the four groups, ovarian atrophy, persistent follicle, persistent corpus luteum and ovarian cyst (Waanga and Janowski, 2000). Therefore, the objective of this study was to investigate the relationships among milk production, glucose, urea nitrogen, phosphorus, BHBA, total protein and progesterone and length of the calving interval.

Materials and methods This study was conducted on 182 (36 normal heat, 146 anoestrous) lactating Holstein cows from June 2006 to February 2007 in a commercial dairy herd in Iran. Cows showed anoestrus to 50-60 days after calving, according to rectal palpation findings were divided into four groups including ovarian atrophy, persistent follicle, persistent corpus luteum and ovarian cyst. Cows were fed according to NRC (2001) to meet production requirements. They were fed a total mixed ration with approximately 19% CP, 1.67 NE_L (MJ/Kg) and 23.6 DMI (Kg/day) for lactating cow (33 kg milk / day). Blood samples were collected once weekly (Friday) at days 50-60 postpartum. Blood samples were collected from tail vein by venoject tubes and centrifuged (3000 × g, 15 min) within 20 minutes after collection and serum was stored at -20c°. Blood serum glucose, urea, phosphorus, total protein and BHBA concentration were measured by spectrophotometer. Statistical procedures were performed using the computational software of SAS. Data for blood serum glucose, urea, phosphorus, total protein and BHBA were analysed by the general linear models (GLM).

Results Blood glucose concentration in normal cows, persistent corpus luteum and ovarian cystic cows had significant differences (P<0.01) between cows with ovarian atrophy and cows with persistent follicles. Blood total protein, although in all cows was in the normal range, its level in persistent follicle and cystic cows was higher than others. Urea levels in normal cows were lower than anoestrus cows (P<0.01). Blood phosphorus concentration in normal cows, persistent corpus luteum and ovarian atrophy were significantly different (P<0.05) from cows with with persistent follicles and cows with ovarian cysts. Blood BHBA concentrations did not differ between normal and anoestrus cows .

Table 1 Least square means by reproductive status for blood glucose, protein, urea, phosphorus and BHBA

	No	Glucose mg/dl	Total protein G/dl	Urea mg/dl	Phosphorus mg/dl	BHBA mol/l
Normal cows	36	53.13 ^a	7.46 ^{ab}	24.39 ^c	5.18 ^{ab}	1.14
Ovarian atrophy	36	39.69 ^c	7.48 ^{ab}	30.45 ^b	6.15 ^a	1.19
Persistent follicle	37	45.58 ^{bc}	7.83 ^a	28.18 ^b	5.17 ^b	1.08
Persistent cor.lut.	37	54.37 ^a	7.1 ^b	33.59 ^{ab}	5.35 ^{ab}	1.17
Ovarian cyst	36	50.58 ^{ab}	7.79 ^a	34.69 ^a	5.12 ^b	1.15

Non-similar letters: significant effect at P<0.05.

Conclusion The present study indicates that post parturition anoestrus are associated with low blood concentrations of glucose, phosphorus and total protein and or increasing of protein, urea and BHBA. Decrease of anoestrus prevalence in dairy cows requires management of rations from precalving to pregnancy, because the timing of negative energy balance has been implicated in the timing of first ovulation.

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Effect of dietary methionine concentration on some blood metabolites of early lactating Holstein cows

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Introduction One possible way to reduce urinary nitrogen (N) excretion is reducing the amount of dietary crude protein fed to dairy cows. In fact, decreasing dietary crude protein (CP) concentration from 184 to 151g/kg, linearly decreased urinary N expressed as gram per day or as a percentage of N intake (Broderick, 2003). Balancing diets for limiting amino acids could improve cow responses fed lower protein diets without increasing N losses in urine. High concentrations of methionine and lysine during early lactation may reduce the risk of metabolic disorders (Berthiaume *et al.*, 2006). A number of blood metabolites are useful as monitors for signs of metabolic disorder. The aim of this experiment was to determine the effect of altering the dietary methionine concentration on some blood metabolite concentrations of early lactating Holstein cows.

Materials and methods Two iso-nitrogenous and iso-energetic diets (CP: 167 g/kg DM; metabolizable energy (ME): 1200 MJ/kg DM) with different methionine to total amino acid ratios as high methionine (HM, 0.062) and low methionine (LM, 0.054) were provided. The diets were offered as total mixed ration for *ad-libitum* intake twice a day to twelve multiparous and four primiparous lactating Holstein cows, averaging 10±4 days in milk and 37.4±4.2 kg/d milk yields, for seven weeks. Blood samples were collected into heparinised tubes from the jugular vein of each cow at 0, 2 and 4 h after the morning feeding in weeks 3 and 6. Samples were centrifuged (3500 rpm, 10 min) and plasma were analysed for glucose, urea-N, insulin, glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT). Data were analysed using the GLM procedure of SAS (2004) in a completely randomised design with dietary methionine concentration as treatment. The statistical model was $Y_{ijk} = \mu + T_i + \epsilon_{ijk}$, where Y_{ijk} = Dependent variable, μ = The overall mean, T_i = Treatment effect and ϵ_{ijk} = Error.

Results Blood concentrations of early lactating Holstein cows are shown in Table 1. There was a significant difference between diets for GOT at week 6 in samples taken at 4 h after feeding ($P < 0.05$).

Table 1 Blood metabolite and liver enzyme concentrations of early Holstein cows fed diets containing different ratios of methionine: total amino acids.

Blood metabolites	Sampling (week)	Time (hours after the morning feed)	Methionine/Total amino acid*		Treatment effect	
			HM	LM	s.e.m.	P
Glucose (mg/dl)	3	0	59.37	60.62	2.62	0.741
		2	55.87	56.87	3.00	0.817
		4	62.37	60.50	2.41	0.591
	6	0	63.37	64.25	2.29	0.791
		2	58.87	61.37	2.52	0.495
		4	62.87	65.25	0.52	0.315
Urea nitrogen (mg/dl)	3	0	16.62	17.87	1.03	0.409
		2	16.87	17.87	0.92	0.457
		4	16.12	17.62	0.76	0.184
	6	0	14.50	17.50	1.17	0.092
		2	15.12	18.25	1.29	0.110
		4	14.37	17.25	1.02	0.067
Insulin (μ IU/ml)	6	0	8.85	9.10	1.53	0.912
		4	9.47	15.75	2.65	0.147
Glutamic oxaloacetic transaminase (U/L)	6	0	74.25	88.75	7.21	0.205
		4	75.00	93.5	5.27	0.047
Glutamic pyruvic transaminase (U/L)	6	0	27.00	26.25	3.23	0.875
		4	24.27	25.25	1.18	0.774

s.e.m.: Standard Error of Mean, P : Probability; * HM: high methionine/ total amino acid, LM: low methionine/ total amino acid

Conclusions Results of the present experiment indicated that various blood metabolites and liver enzymes were not significantly influenced by dietary methionine concentration (except for GOT in week 6 at 4 h after feeding). Results of the present study confirmed the finding of Berthiaume *et al.* (2006), which indicated that a diet supplemented with methionine did not affect blood glucose and liver enzyme concentrations.

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Effect of days dry and diet combination on first 60 days milk production and somatic cell counts of Iranian high producing Holstein cows

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Introduction It has been indicated that to achieve maximal milk production, a non-lactation or dry period is necessary between lactations (Rastani *et al.*, 2005). A period of 45 to 60 days, generally, has been recommended for the dry period. Shortening the dry period to less than 60 days has been promoted during the past few years (Gulay *et al.*, 2003). Several experiments designed to examine the effect of reducing the dry period, to approximately 4-5 weeks, have shown no difference in milk production and/or fat corrected milk in the subsequent lactation (Gulay *et al.*, 2003). The object of the present experiment was to determine the effect of days dry (30-45 vs. 45-60 days) and diet combination on the first 60 days actual milk production, milk fat and protein concentrations, somatic cell counts (SCC), body condition score and conception rate per first insemination of high producing cows.

Material and methods Sixty-six multiparous Holstein cows were selected from a herd with 400 milking cows. All cows had a single foetus and less than 390 days of milk at pre-partum. Animals were assigned randomly in two groups based on dry period length of 30-45 days (Mean: 37; D37) and 45-60 days (Mean: 56; D56). The experiment was carried out from January to September, 2007. At assignment, parity of cows ranged from 2 to 6, body weight and body condition score ranged from 680-750 kg and from 3 to 4.1, respectively. Cows in D56 were dried off 60 days before expected calving and moved to a far-off dry herd (30-60 days before calving) with dry matter intake of 3 kg lucerne, 5 kg maize silage, 1.5 kg wheat straw and 3 kg far-off concentrate (CP: 62 g/kg; ME:12.4 MJ/kg). The cows were moved to the close-up calving group, around 30 days before calving. Cows in D37 were dried off 38 days before expecting the calving and moved directly to the close-up dry herd. The close-up dry ration (DM basis) was consisted of 2 kg lucerne, 6.1 kg maize silage, 1.5 kg wheat straw, 5.5 kg concentrate (CP: 176 g/kg; ME: 13.2 MJ/kg), and 400 g of Anionic salts. After parturition, all cows were fed a total mixed ration (as DM) based on 20% lucerne, 20% maize silage and 60% concentrate. This ration met the requirements of high producing cows (CP: 180 g/kg; ME: 12.1 MJ ME/kg DM). During the first 60 days of lactation, weekly milk production was recorded and milk samples were selected on the same day of each week. Samples were analyzed for fat, protein and SCC using standard procedures. All cows have been subjected to a presynch-ovsynch protocol around 14-24 days post-partum. Cows received two set-up injections of PGF_{2α}, 14 days apart. Animals received GnRH, 12 days after the second injection of PGF_{2α}, followed by a third injection of PGF_{2α}, 7 days later. Cows received a second injection of GnRH 48 h after the third PGF_{2α}, and received blind artificial insemination at 16 h later. Conception rate to first insemination of all cows was recorded. Data were analyzed in a completely randomized design of GLM procedure of SAS (1999), included the treatment effect of dry period with 305 days milk production and SCC of last parity as covariates.

Results No significant effect of dry period length on first 60 days of milk production, milk fat and protein concentrations, and milk SCC was detected (Table1). Mean BCS of cows (D56=2.90, D37=2.92), at first 60 days of post-partum, did not differ due to dry period length. No significant effect of dry period on conception rate per first insemination (28.1% for D56 and 27.9% for D32) was also recorded.

Table 1 Milk production, milk fat and protein concentrations, and milk SCC of cows at first 60 days of post-partum

Item*	dry period length (days)		s.e.m.	P- value
	30-45 (Mean= 37)	45-60 (Mean= 56)		
Milk yield (kg/d)	45.1	44.9	1.56	0.93
Fat (g/kg)	2.9	2.9	0.14	0.88
Protein (g/kg)	2.5	2.5	0.11	0.87
SCC (×1000)	187.3	270.6	91.40	0.52

Conclusions Results of the current study showed no evidence that shortening the dry length to 30-45 (Mean= 37) caused either a negative or positive effect on first 60 days milk production and SCC. However, the range of SCC among the cows was considerable (s.e.m.= 91.40). Results of the present experiment suggested that if an adequate BCS can be achieved before drying off (> 3, < 4.1), there was no advantages of providing cows with a 45-60 d dry period compared with a 30-45 d dry length. These findings support the results obtained by Gulay *et al.* (2003) which reported shorter dry period did not negatively influence subsequent lactation performance compared with cows provided with 60 d dry length. In conclusion, present observations indicated that the short dry off protocol (Mean= 37 days) might be a profitable management practice for dairy farmers, as cows might be kept in milking more days during their life time in a herd.

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Effects of pre- and postpartum feeding fish meal on blood metabolites in early lactating Iranian Holstein cows

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Introduction The transition phase, typically defined as 3 wk before to 3 wk after parturition, is viewed as a critical time in the lactation cycle of a dairy cow. During early lactation dry matter intake (DMI) lags behind the nutrient requirements for milk yield. So, the onset of lactation in the dairy cow is characterized by a dramatic increase in the nutrient demands for milk synthesis. Fish meal (FM) is used in dairy cow ration as a source of RUP in some countries. Greater rumen escape of FM protein is known to increase efficiency of protein utilization in lactating cows. Moreover, FM has an excellent profile of amino acids and is a good source for the 2 most limiting amino acids for milk synthesis, lysine and methionine. Fish meal also contains oil (8 – 10% of DM) with relatively high concentrations of two polyunsaturated fatty acids (PUFA) of the n-3 family, eicosapentaenoic acid (EPA, C20:5) and docosahexaenoic acid (DHA, C22:6), which can be supplied only by the diet because EPA and DHA cannot be synthesized de novo in mammalian systems. Of the available feedstuffs that are high in undegradable protein, fish meal often is effective in improving milk production. Meanwhile, reports on the effect of fish meal on blood metabolites in pre- and postpartum cows are rare and needs more investigation. The objective of this experiment was to compare diets with or without fish meal from transition period up to 35 days in milk (DIM) and their effects on blood metabolites in early lactation Iranian Holstein cows.

Materials and methods From approximately 3 weeks before calving to 35 days after calving, ten multiparous Holstein cows were blocked by parity, expected calving date, and previous 305-d milk production and were randomly assigned within block to 1 of 2 diets containing none (n=5) or 3.5% and 1.95% Kilka fish meal during the prepartum and postpartum periods, respectively. Diets were formulated to be isoenergetic and to provide similar amounts of net energy lactation (NEL) and non-fibrous carbohydrate (NFC) using the Cornell Net Carbohydrate and Protein System. Using vacutainer tubes, blood samples were collected weekly after calving via venipuncture of coccygeal vessels before the morning feeding to monitor serum metabolites. The data were analyzed using the MIXED procedure of SAS (2001) for a completely randomized design with repeated measures.

Results The effect of experimental diets on some blood metabolites during the first 35 DIM are shown in Table 1. The blood metabolites were all similar among the groups. Except for cholesterol, the effect of time was not significant. In contrast with our study, another study showed that feeding fish oil to dairy cows from prepartum to early lactation reduced plasma concentration of glucose (Mattos *et al.*, 2004). Serum urea nitrogen (SUN) was similar among the diets in agreement with other report (Heravi Moussavi *et al.*, 2007a). Dietary CP content is the most important nutritional factor influencing blood and milk urea nitrogen. It seems because of adjusting the diets to have close amount of metabolizable protein in the current study, SUN was similar among the diets. Results of the current study based on the similar serum triglycerides (TG) level showed that the diets had no apparent impact on fat metabolism. The diet had no effect on serum cholesterol in consistent with another recent report (Heravi Moussavi *et al.*, 2007b). The effect of time was significant ($P < 0.001$) and cholesterol concentrations increased after parturition. Feeding fat to dairy cattle sometimes increased plasma cholesterol, and the increase is independent of the degree of fatty acid saturation, although some other study reported no difference.

Table 1 Serum metabolite concentration during the first five weeks postpartum in cows fed diets containing none (Control) or 3.5 and 1.95% fish meal during prepartum and postpartum periods (Supplemented)

Parameter	Diets		SED	Treat. P Value	Time P Value
	Control	Supplemented			
Glucose, mg/dl	51.0	51.52	2.31	0.87	0.12
Serum urea nitrogen (SUN), mg/dl	15.13	14.0	0.9	0.66	0.18
Triglycerides, mg/dl	27.84	29.52	4.37	0.79	0.38
Cholesterol, mg/dl	155.10	145.66	7.0	0.37	<0.01

Conclusions The results of this study demonstrate that adding fish meal to the diet did not exert general metabolic effects as plasma concentrations of glucose, SUN, cholesterol as well as serum TG were similar among dietary groups.

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Canola meal as a substitute for soybean meal in diet of early lactation Iranian Holstein cows

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Introduction Canola meal is one of the most widely used protein sources in animal feeds. It has an excellent amino acid profile and it is rich in vitamins and essential minerals. Many experiments have been conducted on the value of canola meal for dairy cows. Excellent and often improved milk production has resulted from the use of canola meal as a main protein source. Canola meal provides an important contribution to both rumen microbial protein needs as well as to the digestible amino acids required for animal growth and lactation. Canola meal possesses an excellent RDP profile that may stimulate microbial growth in the rumen. Although canola meal was extensively degraded in the rumen, its 12-h residue still provided an estimated AA profile to the intestinal tract that was close to the AA profile of milk protein (Piepenbrink and Schingoethe, 1998). Kendall *et al.* (1991) found that the effective degradability of canola meal averaged 51.5%. This compared to 59.1% for soybean meal. In the current animal feed market in Iran, canola meal is cheaper than soybean meal (320 vs. 482 Toman/kg; US \$1=933 Toman). The objective of this experiment was to evaluate substitution of soybean meal with canola meal and measure its effects on milk production and composition, and dry matter intake in early lactation Iranian Holstein cows.

Materials and methods From d 5 to 56 postpartum, cows were fed diets that were isoenergetic containing soybean meal (SBM; n = 5) or canola meal (CM; n=5). Holstein cows were blocked in pairs based on their previous 305-d milk, parity (2nd and 3rd to 5th) and expected calving dates. Cows within each block were randomly assigned to one of the two treatments. Cows were housed in tie stalls and fed the TMR two times a day to allow 5 to 10% orts (as-fed basis). The TMR were sampled weekly throughout the experiment and DM content was determined by drying at 110 °C for 18 h. The data were analyzed using the MIXED procedure of SAS (2001) for a completely randomized design with repeated measures. Overall effect of treatment was tested using cow within treatment as the error term. For all analyses, least square means were calculated.

Results Milk production was similar among diets but increased over the time ($P < 0.001$). Diet had no effect on dry matter intake (DMI). The effect of time was significant ($P < 0.001$) and DMI was increased over the time. Except than fat content, milk composition was similar among the dietary groups. Canola meal increased milk fat ($P = 0.049$). Diet had no impact on body weight (BW) and body condition score (BCS). The BW and BCS were decreased by 7 week of experiment ($P < 0.001$).

Table 1 Least squares means of production parameters in cows fed diets containing soybean meal (SBM) or canola meal (CM) during the first 8 weeks postpartum

Parameter	Diets		SED	Treat. P Value	Time P Value
	SBM	CM			
Milk, kg/d	36.51	36.00	0.76	0.64	<0.001
Fat					
%	3.25	3.47	0.07	0.05	0.40
Kg/d	1.21	1.24	0.04	0.66	<0.01
Protein					
%	3.07	3.10	0.04	0.66	<0.001
Kg/d	1.13	1.11	0.04	0.80	<0.01
Lactose					
%	4.60	4.62	0.07	0.88	<0.001
Kg/d	1.70	1.65	0.06	0.56	0.04
DMI, kg/d	20.16	21.03	0.34	0.11	<0.001
BW, kg	579	580	24.12	0.97	<0.001
BCS	2.99	2.96	0.09	0.83	<0.001

Conclusions The results of this study demonstrate that substituting soybean meal with canola meal in the diet of early lactating cows had no effect on dry matter intake, milk production and composition, except than milk fat. So, economically, diets containing canola meal could be better in terms of reducing the diet expenses.

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Effect of diets containing soybean meal or canola meal on follicular dynamic in early lactation Iranian Holstein cows

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Introduction Although there is no clear evidence to fully describe the mechanism involved in glucosinolate-related effects on animal reproduction, lowered fertility in animals fed diets with rapeseed meal (RSM) inclusion is related to glucosinolate (Gls) content in the diet (Mawson *et al.*, 1994). The degree of reproduction impairment depends both on glucosinolate content and on the type of animal. Negative effects of feeding high Gls RSM on fertility and poor reproduction traits in cows fed high amounts of very low-glucosinolate rapeseed meal were reported (Tripathi and Mishra, 2007). Therefore, cows fed diet not only high in Gls RSM but also high in low-glucosinolate RSM are sensitive to dietary glucosinolates. Diets containing Gls may cause thyroid disturbances that depressed fertility (Ahlin *et al.*, 1994), such as increased calving to conception period, more numbers of inseminations per pregnancy and more numbers of acyclic and cystic ovaries (Tripathi and Mishra, 2007). Canola is a trademarked quality description of a group of cultivars of rapeseed variants from which low erucic acid rapeseed oil and low glucosinolate meal are obtained. In the current animal feed market in Iran, canola meal is cheaper than soybean meal (320 vs. 482 Toman/kg; US \$1=933 Toman). The objective of this experiment was to evaluate substitution of soybean meal with canola meal and measure its effects on follicular dynamic and days postpartum to first ovulation in early lactation Iranian Holstein cows.

Materials and methods From days 5 to 56 postpartum, cows were fed diets that were isoenergetic containing soybean meal (SBM; n = 5) or canola meal (CM; n=5). Holstein cows were blocked in pairs based on their previous 305-day milk yield, parity (2nd and 3rd to 5th) and expected calving dates. Cows within each block were randomly assigned to one of the two treatments. Ultrasound measurements of follicular activity were made on alternate days from days 10 - 35 postpartum (PP) to ascertain the characteristics and fate of the first follicular wave utilising a 7.5-MHz rectal transducer. Dominant follicle development was characterised by follicular mapping of recorded ultrasound images. Follicular recruitment during the first follicular wave after parturition was evaluated by quantification of the numbers of 5 to 10-mm follicles on d 10 and 14 PP. A dominant follicle was defined as a follicle that was >10 mm in diameter in the absence of other large (>9 mm) growing follicles. The data were analysed using the General Linear Model (GLM) procedure of SAS (2001) for a completely randomised design.

Results Diet had no effect on follicular parameters and days postpartum to first ovulation (Table 1). The number of medium-sized follicles (5 to 10 mm) present on days 10 and 14 PP, diameter of the first dominant follicle on days 10 and 14 PP, maximum diameter of the first dominant follicle and number of days until detection of a follicle \geq 10 mm in diameter were similar among diets. Except for 3 cows, the others were ovulated. The mean and median days to first PP ovulation were not affected by the diets.

Table 1 Follicular dynamic parameters in cows fed total mixed rations containing soybean meal (SBM) or canola meal (CM)

Parameter	Diet		SE	P
	SBM	CM		
Number of follicles (\geq 5 mm in diameter) on day 10	2.00	2.60	0.47	0.40
Number of follicles (\geq 5 mm in diameter) on day 14	2.83	3.00	0.56	0.83
Follicles diameter (\geq 5 mm) on day 10, mm	10.75	9.80	1.79	0.72
Follicles diameter (\geq 5 mm) on day 14, mm	12.00	12.41	1.21	0.81
Diameter of first dominant follicle, mm	16.16	19.60	1.35	0.11
Days postpartum to first dominant follicle, d	14.50	16.40	0.96	0.20
Days postpartum to first ovulation, d	17.60	20.25	1.35	0.21

Conclusions The results of this study demonstrated that substituting soybean meal with canola meal in the early lactation cows had no apparent effect on follicular dynamics and days postpartum to first ovulation. So, economically, diets containing canola meal could be better in terms of reducing the dietary costs.

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Effect of alfalfa hay particle size and dry matter content of barley based diets on ruminal, faecal and blood measurements of dairy cows in early lactation

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Introduction Selective consumption (sorting) of a diet by lactating dairy cows can cause the cows to ingest a diet that could differ from the diet offered (Leonardi and Armentano, 2003). There are several techniques available to minimize the sorting of diets by dairy cows including addition of water to the diet or applying appropriate forage processing. The objective of this experiment was to understand if particle size and dry matter (DM) content of the diet affected health and performance of dairy cows.

Materials and methods This experiment was conducted at the Ferdowsi University of Mashhad Dairy Research Farm. Eight multiparous Holstein cows in early lactation (28±12 days in milk) with daily milk production of 43±3.5 kg/d were allocated to four treatments in a change-over design with 4 periods of 21d. Cows were housed individually in tie-stalls and fed *ad-libitum* twice daily. The balanced diets (NRC, 2001) had the same chemical composition. Diet main ingredients were as follows (g/kg): lucerne hay, 200, maize silage, 150, barley grain, 310, cottonseed, 90, soyabean meal, 120, safflower meal, 60, wheat bran, 30 and protected fat, 20. Two particle sizes of lucerne hay (5 and 20 mm theoretical cutting size) and two levels of TMR DM (without and with water addition up to 0.50 of DM) were applied as experimental treatments. Water was sprinkled onto the diet daily during diet preparation. Feed and orts samples were collected during the last week and pooled by animal in each period. The amount of feed offered was adjusted daily to obtain around 0.10 orts (as fed basis). Particle size of TMR and orts was determined by the ASAE (1992) particle size separator. Blood samples were taken 4hrs after the morning feed and analyzed for pH and blood gases (Radiometer ABL50). Data were analyzed using the mixed model procedure of SAS.

Results Both lucerne hay particle size reduction and water addition to the diet resulted in significant changes in TMR particle size distribution (Table 1). Reduction of diet particle size caused a significant decrease in total chewing time (TC), although it had no effect on ruminal pH (Table 2). Conversely water addition resulted in a significant reduction in rumen pH (P=0.02). Blood pH and bicarbonate were not affected by any treatment. In addition treatments had no effect on faecal pH or rumen ammonia nitrogen concentration. (Table 2).

Table 1 TMR particle size distribution (g/100g DM), eating time and total chewing time of the experimental cows

Screen size (mm)	Long hay		Short hay		SEM	Effect ¹		
	Dry	Wet	Dry	Wet		PS	DM	PS×DM
19	1.13	2.57	0.59	0.44	0.18	<0.01	0.02	<0.01
12.7	3.24	2.71	0.96	1.27	0.27	<0.01	0.20	<0.01
6.3	24.51	21.68	18.96	18.27	0.56	<0.01	0.70	<0.01
3.96	11.37	13.52	10.72	13.65	0.46	0.07	0.03	0.30
1.18	42.36	46.59	46.62	52.62	0.56	<0.01	<0.01	Ns
Pan	17.53	13.11	22.32	13.94	0.51	<0.01	<0.01	0.02
Pef ²	82.60	87.08	77.85	86.26	0.51	<0.01	<0.01	0.02
peNDF _{1.18} ³	31.39	33.96	30.36	35.29	2.15	0.41	0.48	0.65

¹ PS hay particle size, DM, TMR dry matter.

² pef Physical effectiveness factor: cumulative proportion of particles (DM basis) retained by ASAE sieves above pan

³ peNDF_{1.18} Physically effective NDF estimated as the NDF content of TMR multiplied by pef.

Table 2 Ruminal, blood and faecal measurements and eating and ruminating time of the cows

	Long hay		Short hay		SEM	Effect		
	Dry	Wet	Dry	Wet		PS	DM	PS×DM
pH								
Rumen	6.22	5.93	6.18	5.96	0.08	0.61	0.02	0.35
Blood	7.26	7.27	7.26	7.28	0.01	0.93	0.28	0.83
Faeces	6.80	6.80	6.78	6.78	0.08	0.83	0.80	0.41
Blood HCO ₃ ⁻ (mmol/L)	20.9	21.1	21.1	21.57	0.92	0.32	0.19	0.33
Rumen N-NH ₃ (mg/dl)	18.1	18.3	19.1	17.2	1.82	0.88	0.33	0.34
ET (min/kg peNDF _{1.18})	47.57	42.83	48.84	44.07	2.73	0.43	0.02	0.52
TC (min/d)	816.3	806.9	747.5	735.0	50.98	0.03	0.25	0.46

Conclusion. Rumen pH reduction due to water addition may be explained by reduction of eating time (ET) per kg of NDF and peNDF_{1.18} and consequent decrease in saliva secretion (Table 2). The other part of rumen pH reduction can be attributed to the attachment of diet fine particles (mainly rumen degradable organic matter) to the coarser parts and their utilization a few hours after feeding by the cows.

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Effect of feeding protected fat on dairy cow productivity and fertility

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Introduction Rumen protected fats are often included in dairy cow rations in order to increase the energy density of the ration without compromising rumen function. Various studies have examined the effects of protected fats, with some studies reporting an improvement in various fertility parameters (McNamara *et al.*, 2003). This study examined the effect of feeding protected fat (Megalac™) on production parameters, and on the reproductive performance of high-yielding Holstein-Friesian dairy cattle.

Materials and methods Eighty nine Holstein cows (rolling 12 month average milk yield of 9,561 kg at 3.77 % butterfat and 3.2 % protein) were grouped into two blocks on the basis of calving date, previous yield and lactation number. Lactating cows were offered a semi-TMR mixture of grass silage, wholecrop wheat silage, brewers grains, molasses and 7.5kg of treatment concentrates (for maintenance plus 28 litres) with standard concentrates fed to yield in the parlour at a rate of 0.4kg per litre of milk above 28 litres. At calving, individual animals were allocated at random to one of the following treatments: (C) Control (no fat supplementation), or (M) TMR concentrate pellet formulated to supply 600g/cow/day calcium salt of palm fatty acids (Megalac™). Milk yield was recorded at each milking, with samples from each individual cow being taken once per month at pm and am milking for butterfat and protein analysis (CIS, Paisley, UK). Further milk samples were taken three times a week from calving until 60 days post-partum for milk progesterone analysis (Ridgeway Science Ltd.). Fertility data were recorded using Interherd (NMR). Statistical analyses were performed using Linear Mixed Effect models allowing for repeated measures (milk yield), Chi-squared analysis for proportional data and ANOVA.

Results The M group produced an average of 0.65 kg more milk/cow/day during weeks 1-7 of lactation, and 0.98kg more milk/cow/day during weeks 8-30 of lactation than the control group, although these differences were not statistically significant ($P > 0.05$). There were no significant differences between the two groups in estimated total (M vs. C; mean \pm 95% CI 10,060kg \pm 815 vs. 9,831 \pm 630 kg) or estimated 305 day-adjusted lactation yields (M vs. C; 9,487 \pm 699 kg vs. 9,387 \pm 587 kg) ($P > 0.05$). Similarly, milk butterfat concentrations, milk protein concentrations and total solids yields were not significantly different between the groups ($P > 0.05$). Metabolic profile analysis demonstrated no significant differences in energy (β -hydroxybutyrate, NEFA and glucose) or protein (urea-N and albumin) status between the two groups in the first month of lactation. There were no significant ($P > 0.05$) differences between groups in the time to first rise in milk progesterone post-partum (M vs. C; median and IQ range; 31 days (22-43) vs. 26 (19-38) days, or percentage of normal ovarian cycles (M vs. C; 59% vs. 63%) as measured using milk progesterone levels. The effects on other major fertility parameters are shown in Table 1. Control conception rate was low during the trial. Supplementation with protected fat increased ($P < 0.05$) conception rates, increased 100-day in-calf rate, and reduced 200-day not-in-calf rate ($P < 0.01$) compared to control.

Table 1 Effect of feeding protected fat (Megalac™) on fertility parameters

	Megalac Treatment	Control	Significance
Number of cows (excludes cull cows)	40	42	
Calving to 1 st visible oestrus interval [mean (\pm 95% CI)]	65.6 days (\pm 6.61)	65.4 days (\pm 6.16)	NS
Calving to 1 st service interval [mean (\pm 95% CI)]	74.1 days (\pm 4.56)	73.8 days (\pm 4.32)	NS
Number of services per conception	2.1	2.5	NS
Conception rate (1 st service)	32.5%	14.3%	$P < 0.05$
Conception rate (all services)	39.3%	21.2%	$P < 0.05$
100 day in-calf rate (%)	18 (45%)	9 (21.4%)	$P < 0.05$
200 day not-in-calf rate (%) (including not in calf at end of service period)	6 (15%)	19 (45.2%)	$P < 0.01$
Calving to conception interval [median (interquartile range)]	99 days (75 – 142)	118 days (77.5 – 164)	NS
Number of cows with cysts (%)	2 (5%)	4 (9.5%)	NS
Number of fertility treatments per cow	1.15	1.19	NS

Conclusions The results of this trial showed a tendency for increased milk yield by supplementation with Megalac™. However, there were marked improvements in fertility in the treatment group compared to the controls. These fertility effects appeared to be independent of metabolic status (as measured using metabolic profiles) and ovarian function (as measured using milk progesterone assay). This improvement in fertility is therefore likely to be a result of improved quality and developmental competency of the oocyte following fat supplementation.

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Effect of a dietary cellulase/xylanase enzyme mixture on dairy cow performance

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Introduction An increase in the input costs in the dairy industry has demonstrated the need for methods to increase production efficiency. One way of increasing efficiency would be to increase the bioavailability of nutrients in a feed stuff, which might be accomplished through the direct feeding of enzymes (Yang *et al.* 1999). The use of enzymes as additives in ruminant diets has received considerable research interest recently following positive responses observed in feeding trials (Lewis *et al.* 1995; Beauchemin and Rode, 1996). The objective of present study was to determine the effects of an enzyme mixture (Natuzyme, Bioproton Pty Ltd, Australia) on dairy cow performance.

Materials and methods Nine Holstein cows in early lactation were randomly assigned to treatments in a 3×3 Latin square design. Cows averaged 53.88±8.19 days in milk. Experiment was in 3 periods and each period was 21 days. Treatments were: no enzyme, 0.5 kg enzyme mixture / tonne concentrate portion of the TMR and 1.0 kg enzyme mixture / tonne concentrate portion of TMR. The enzyme mixture (from *Trichoderma Longibrachiatum*) contained phytase, beta-glucanase, alpha-amylase, cellulase, pectinase, xylanase and protease activities. The cows were given ad libitum a TMR composed (g/kg) of 340 forage (200 lucerne, 140 maize silage) and 660 concentrate (250 barley, 80 maize, 70 cottonseed, 60 soyabean meal, 90 cottonseed meal, 50 beet pulp, 43 bran, 2 urea, 5 lime, 2 premix, 2 salt, 6 fat meal), based on NRC (2001). Data were analysed using the General Linear Models procedure of SAS V6.12 for ANOVA to evaluate differences among experimental groups; means were compared with Duncan's test.

Results Milk production, protein, lactose, and digestibility of dry matter were higher in cows fed enzyme-treated diets compared to those fed the control diet, but these differences were not significant. Dry matter intake was significantly higher for the control diet ($P<0.05$) and feed efficiency therefore was significantly greater in treatment groups ($P<0.05$, Table 1).

Table 1 Effects of enzyme on cows performance

Treatment	No enzyme	Enzyme (0.5 kg/tonne)	Enzyme (1.0 kg/tonne)	SEM
Dry matter intake(kg/d)	25.56 ^a	24.02 ^c	24.76 ^b	0.02
Milk production(kg/d)	32.86	33.2	34.34	0.65
Protein (g/kg)	33.4	33.7	33.5	0.1
Lactose (g/kg)	46.4	46.5	46.6	0.5
Fat (g/kg)	35.9	34.0	36.4	1.4
Dry matter digestibility (g/kg)	782	804	790	0.3
Feed efficiency	1.29 ^b	1.38 ^a	1.38 ^a	0.02

Means in the same row with different letters are different ($P<0.05$)

Conclusions Applying an enzyme mixture to concentrates prior to feeding, increased feed efficiency (4). So, data showed that the enzyme can improve dairy cow performance.

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Effect of supplemental fat and NDF on fibre digestibility, ruminal pH and chewing activity in lactating dairy cows

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Introduction The minimum amount of dietary NDF needed by a cow is largely based on ruminal and animal health (NRC, 2001). On average, NDF is less digestible than non-fibre carbohydrates (NFC, NRC, 2001). Therefore, NDF concentration is negatively correlated with energy concentration. The NDF is related to ruminal pH, fat percentage, and chewing activity (NRC, 2001). Fat is commonly added to diets of high yielding dairy cows to increase energy density. However, the addition of fat can interfere with rumen fermentation which can result in decreased fibre digestion. Fibre characteristics of the diet may influence the extent to which fats interfere with rumen fermentation. Therefore, the type of fibre influenced the degree of negative effects caused by ruminally active fats (Tackett *et al*, 1996). The main aim of the present research was to evaluate the effect of supplemental fat and NDF on fibre digestibility, rumen pH and chewing activity in lactating dairy cows.

Material and methods Eight multiparous Holstein dairy cows were used based upon a 2x2 factorial experimental design. Treatments were: 1) 320 g NDF/kg DM and no added fat; 2) 320 g NDF and 25 g fat /kg DM 3) 420 g NDF /kg DM and no added fat and 4) 420 g NDF and 25 g fat /kg DM. The source of fat was cottonseed oil, and cottonseed hulls (CSH) replaced with barley to increase dietary NDF. Diets were formulated (NRC, 2001) to contain 170 g CP /kgDM CP and 6.90 MJ NE₁ /kg DM. The diets were fed *ad libitum* as a total mixed ration (TMR) twice daily with a concentrate to forage ratio of 60:40. The digestibilities of DM, OM, CP, NDF, and ADF were determined by direct method (faeces collection), ruminal pH by two methods (stomach tube and rumenocentesis) and chewing activity monitored visually for a 24 hour period. Data were analyzed using the general linear model (GLM) procedure of statistical analysis system (SAS) and treatment means were compared with each others using Duncan multiple range test.

Results The digestibilities of NDF and ADF were significantly higher in diets with high NDF (P<0.05). Ruminal pH was lower in samples taken by rumenocentesis compared with samples taken by stomach tube, probably due to lower contamination of rumen fluid taken by rumenocentesis. The results also indicated that cows spent a similar time eating, ruminating and total chewing time (eating+rumination) whether expressed per day or per kg of DMI.

Table 1 DMI, ruminal pH, digestibilities of NDF and ADF and chewing activity in diets with different levels of NDF and fat

Item	Diets				SEM
	320 g NDF/kg DM +No fat	320 g NDF + 25 g fat /kg DM	420 g NDF/kg DM +No fat	420 g NDF + 25 fat g/kg DM	
DMI (kg/d)	24.43	23.63	24.95	24.71	0.320
pH (stomach tube)	6.60	6.62	6.65	6.82	0.063
pH (rumenocentesis)	5.95	6.01	6.14	6.10	0.058
NDF digestibility (g/kg)	550.50 ^{ad}	530.46 ^a	570.28 ^{bc}	560.17 ^{cd}	0.258
ADF digestibility (g/kg)	520.51 ^{ad}	510.00 ^a	580.16 ^{bc}	560.13 ^{cd}	0.176
Total chewing time l:					
Min/day	702.37	680.75	668.37	704.50	0.789
Min/kg DMI	28.91	29.11	26.82	28.61	0.188

Values with different superscripts in each row were significantly different (P<0.05) from other values.

Conclusion The results obtained from the present study showed that NDF provided by cottonseed hulls (as a source of non forage fiber) had higher digestibility most likely due to superior digestibility of by-products with high fibre as compared to forages (Sarwar *et al*, 1992). Chewing activity was not affected by the different diets probably due to its smaller particle size. Furthermore, fat had a less detrimental effect on fibre digestion in diets with high NDF. Therefore, it could be suggested that a part of ration concentrate could be replaced by non forage fibre sources which in turn would decrease ration cost.

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Nitrogen utilisation and manure nitrogen output for Jersey-Holstein and pure breed Holstein dairy cows

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Introduction The European Union Nitrates Directive stipulates mandatory measures that must be included in national action programmes, one of which involves a limit on the amount of livestock manure which may be applied to land each year, set at 170 kg organic nitrogen (manure N) per hectare. This limit will have very significant implications for stocking rates on intensive livestock farms. Consequently, there is increasing interest in developing mitigation strategies to reduce N output in faeces and urine in animal production. The objectives of the present study were to evaluate the effects of cross-breeding of Holstein cows with Jersey sires on the efficiency of N utilisation and manure N output.

Materials and methods A 4 period changeover study was undertaken to compare the efficiency of N utilisation between Holstein (n = 8) and Jersey-Holstein (n = 8) first lactation cows offered *ad libitum* mixed diets of grass silage with 2 levels of concentrate (0.30 vs. 0.70 kg/kg DM). The levels of concentrates in diets offered to each breed were of either 0.30, 0.70, 0.30 and 0.70 kg/kg DM or 0.70, 0.30, 0.70 and 0.30 kg/kg DM during the 4 periods (6 weeks/period). The animals were on experiment from 28 days of lactation and there was a 10 week break interval between periods 2 and 3. During the pre-experimental period and the break interval, all animals were offered the same silage and concentrates with a ratio of 0.50/0.50 (DM basis). The animals were housed in cubicle accommodation and then transferred to metabolism units on the 11th day from the end of each period. Faeces and urine outputs were collected for 6 days when animals were housed in the metabolism units. Data were analysed by ANOVA to examine effects of breed and dietary concentrates level using experimental period as block.

Results There was no significant treatment interaction on N utilisation or manure N output (Table 1). As expected, the high concentrate level produced a significantly higher N intake and outputs in faeces, urine and milk and N retention ($P < 0.01$ or 0.001), irrespective of cow breed, and also a higher proportion of manure N output over DM intake and GE intake ($P < 0.001$). Dietary concentrate level had no significant effect on N partitioning between milk and body tissue. On average across the 2 concentrate levels, although Jersey-Holstein cows had a higher N intake ($P < 0.01$), there was no significant difference between the 2 breeds in N partitioning between milk and body tissue as expressed as either total output (g/d) or as a proportion of N intake (g/g). Total manure N output ($P < 0.05$) and manure N/milk yield ($P < 0.01$) were higher with Jersey-Holstein than Holstein cows. However, manure N output as a proportion of energy-corrected milk yield (milk energy output/energy content per kg standard milk) was similar between the 2 breeds. Cross-breeding had no significant effect on manure N output as a proportion of N intake, DM intake or GE intake.

Table 1 Nitrogen utilisation and manure nitrogen output for Holstein and Jersey-Holstein dairy cows

Concentrate level (kg/kg DM)	Holstein		Jersey-Holstein			Conc. L	Significance ¹	
	0.30	0.70	0.30	0.70	s.e.		Breed	
Nitrogen intake (g/d)	417	526	435	565		10.6	**	***
Faecal N output (g/d)	131	160	136	168		6.6		***
Urine N output (g/d)	156	194	166	212		6.9		***
Milk N output (g/d)	92	118	98	123		3.7		***
Retained N (g/d)	38	54	35	62		7.4		**
N digestibility (g/g)	0.69	0.70	0.69	0.70		0.011		
Milk N/N intake (g/g)	0.22	0.23	0.23	0.22		0.007		
Retained N/N intake (g/g)	0.09	0.10	0.08	0.11		0.014		
Manure N output (g/d)	287	355	302	380		9.1	*	***
Manure N/N intake (g/g)	0.69	0.67	0.70	0.67		0.012		
Manure N/DM intake (g/kg)	19.71	21.32	19.93	21.26		0.349		***
Manure N/GE intake (g/MJ)	1.07	1.16	1.08	1.15		0.021		***
Manure N/Milk yield (g/kg)	16.18	16.69	17.25	19.34		0.639	**	*
Manure N/EC milk yield ² (g/kg)	16.20	16.58	15.35	16.69		0.653		

¹ There was no significant interaction on any parameter between cow breed and concentrate level (Conc. L)

* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$

² EC milk yield = energy corrected milk yield (= milk energy output/energy content per kg standard milk)

Conclusions Results of the present study indicate similar N partitioning between milk and body tissue and manure N output as a proportion of N intake, DM intake or energy-corrected milk yield for Holstein and Jersey-Holstein dairy cows.

Effect of dietary phosphorus level on bone composition of dairy cows

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Introduction There is increasing environmental pressure to reduce phosphorus (P) inputs to agricultural systems, including dairy systems. In a recent study (Ferris *et al.*, 2007) dairy cow performance (milk yield, milk composition, food intake and fertility) was found to be unaffected when dairy cows were offered diets containing reduced levels of dietary phosphorus over a four-year period. However, if cows are offered diets containing inadequate dietary P levels for an extended period, bone P reserves may become depleted. The aim of this experiment was to examine the effect of reducing dietary P level on bone composition. Bone samples were collected from cows culled/removed from the study described by Ferris *et al.* (2007).

Materials and methods One hundred winter calving, Holstein Friesian dairy cows were managed on diets containing either 'high' or 'reduced' contents of dietary P (50 animals/treatment) in a four year study. During the winter periods, cows were offered diets containing grass silage and maize silage (approximately 70 : 30 DM basis), supplemented with between 10.0 – 13.0 kg concentrate/cow/day (according to silage quality). During the summer periods (Years 1 and 2) half of the animals grazed both by day and by night, while the remaining animals grazed by day, and were housed by night and offered grass silage. During the summer periods in Years 3 and 4, all animals grazed by both day and night. Concentrate feed levels during the summer periods were either 3.0 or 4.0 kg/day. During both the winter and summer periods, total ration P content was adjusted by modifying the amount of P in the concentrate component of the diet offered. During the winter period, concentrates were formulated to contain either 4.4 or 7.0 g P/kg DM (approx.), while summer concentrates were formulated to contain either 3.6 or 6.8 g P/kg DM (approx.). The concentrate containing the lower P level was formulated without additional mineral P being added, while di-calcium P was added to this concentrate to produce the higher P concentrate. P levels within the higher P concentrates were based on levels measured within commercially available concentrates at the time of the study. The mean P content of the high and reduced P diet was 4.8 and 3.5 g/kg DM (winter period) and 4.2 and 3.5 g/kg DM (summer period), respectively. A 16 mm diameter bone core was removed from the 12th rib (approximately 35 - 40 cm ventral to the spinal process) of 63 cows culled during the course of the experiment. In addition, on completion of the experiment, a bone core was surgically removed from the same position from 15 pregnant cows using a trephine. These rib cores were split, and the soft trabecular bone removed using sandpaper. The remaining cortical bone sample was analysed for oven dry matter, ash, calcium and P concentrations. Treatment effects on bone composition were analysed by ANOVA (REML) as a 2 (dietary P level) x 4 (lactation when culled) factorial design, with the effect of 'lactation when culled' tested for linear trends.

Results Diet had no significant effect on the dry matter, ash or calcium content of bone ($P>0.05$), whereas the P content of bone, when expressed on either a fresh, DM or volume basis (g/l), was significantly lower for cows offered the reduced P diet ($P\leq 0.05$). While there was a significant linear relationship between lactation when culled ($P<0.05$) and the ODM and ash content of bone, lactation when culled had no effect on either the Ca or P content of bone. There were no significant interactions ($P>0.05$) between lactation when culled and dietary P level, for any of the parameters examined.

Table 1 Effect of dietary P level, and lactation when culled, on bone parameters of dairy cows

	Dietary P		SED	Lactation when culled				SED	Significance	
	High P	Reduced P		1	2	3	4		P	Lactation when culled (lin.)
Oven DM (g/kg)	854	852	3.4	848	850	857	857	5.5	NS	*
Ash (g/kg fresh)	586	580	4.2	576	580	586	589	6.7	NS	*
Phosphorus										
-g/kg fresh	105	102	1.2	102	104	105	104	2.0	*	NS
-g/kg DM	123	120	1.2	120	122	123	121	2.0	*	NS
-g/kg ash	179	177	1.7	177	179	180	176	2.7	NS	NS
-g/l	156	151	2.3	150	153	158	153	3.7	**	NS
Calcium										
-g/kg fresh	185	184	7.1	174	183	206	175	11.5	NS	NS
-g/kg DM	216	216	8.0	205	215	241	205	12.9	NS	NS
-g/kg ash	315	318	11.3	302	315	352	298	18.3	NS	NS
-g/l	276	272	11.0	257	271	309	260	17.8	NS	NS

Conclusions While cows offered a reduced P diet had significantly lower bone P concentrations than those on the high P diet, there was no evidence of a progressive depletion in either bone P or bone calcium reserves over a number of lactations. The reduction in bone P reserves observed within the current study is unlikely to have been of practical significance in terms of bone strength.

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The impact of uterine infection on the reproductive performance of dairy cows

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Introduction Management at calving plays an important role in the subsequent reproductive performance of dairy cows. Postpartum involution in cows, for example, can be impaired by infectious microorganisms ascending into the uterus via the vagina, usually as a result of poor hygiene at parturition or insemination, aided by immune suppression or tissue damage. Susceptible cows are usually those which have suffered previously from dystocia, retained placenta, twin birth, stillbirth or metabolic disorder (Kim and Kang, 2003). The aim of this study was to assess the predisposing factors leading to uterine infection in dairy cows and evaluate its impact on the cow's performance.

Materials and methods Performance indicators included feed intake, milk fat and protein composition, milk yield, fertility and culling. Data were studied from 2914 completed lactations from the Holstein Friesian Langhill Dairy herd between January 1990 and August 2005. There were 402 cases of uterine infection in the Langhill herd, a level of 13.8 per cent during the study period. The Langhill herd are on a long-term genetic breeding and feeding systems project. Approximately equal numbers of select line (selected for kilograms of fat plus protein) and control line cows (average genetic merit for fat plus protein production) were offered either a high or low concentrate diet as a total mixed ration (TMR). The first diagnosed presence of uterine infection by the farm veterinarian was used in this study, with suspected cases being identified by oestrus not being observed or evidence of a vaginal discharge. The data were analysed using general linear mixed model as described by Breslow and Clayton (1993) in Genstat Version 7.2 (Lawes Agricultural Trust, 2004) with significance being attributed at P<0.05. Using a chi-squared (χ^2) test, the incidence of calving assistance, conception failure and incidence of culling were each analysed with the occurrence of uterine infection to test their association.

Results Calving assistance was highly associated with the incidence of uterine infection ($\chi^2 = 106.63$, P<0.001). Failure to conceive ($\chi^2 = 3.89$, P<0.05; $\chi^2 = 11.20$, P<0.001) and culling ($\chi^2 = 13.66$, P<0.001, $\chi^2 = 8.55$, P<0.01) were also implicated with calving assistance and uterine infection respectively. Table 1 shows the main predisposing factors related to an incidence of uterine infection and the need for calving assistance.

Predisposing factor	Wald statistic	df	P					
Calving assistance				No assistance	Assisted			
Uterine infection	5.81	1	<0.05	0.11 (0.01)	0.28 (0.02)			
No. of calves born				One	Two			
Assistance	24.45	1	<0.001	0.17 (0.01)	0.32 (0.04)			
Uterine infection	39.46	1	<0.001	0.13 (0.01)	0.39 (0.04)			
Sex				Male	Female			
Assistance	26.97	1	<0.001	0.17 (0.01)	0.09 (0.01)			
Presentation of birth				Normal presentation	Malpresented			
Uterine infection	11.69	1	<0.001	0.13 (0.01)	0.33 (0.04)			
Retained placenta				No retained placenta	Retained placenta			
Uterine infection	100.68	1	<0.001	0.12 (0.01)	0.49 (0.04)			
Dry period liveweight change (kg)				<39	-39 to -15	-14 to 10	>10	
Assistance	18.65	3	<0.001	0.19 (0.02)	0.11 (0.01)	0.10 (0.01)	0.08 (0.01)	
Cow to calf birthweight ratio				<12.41	12.41 to 13.70	13.71 to 15.11	>15.11	
Assistance	13.34	3	<0.01	0.27 (0.02)	0.13 (0.01)	0.07 (0.01)	0.08 (0.01)	
Pre-calving gestation length (days)				<280	280 to 283	284 to 286	>286	
Uterine infection	10.23	3	<0.05	0.16 (0.02)	0.10 (0.01)	0.12 (0.02)	0.14 (0.02)	
Lactation no.				1	2	3	4	≥5
Assistance	112	4	<0.001	0.32 (0.02)	0.10 (0.01)	0.12 (0.01)	0.09 (0.01)	0.13 (0.02)
Uterine infection	10.71	4	<0.05	0.17 (0.01)	0.12 (0.01)	0.10 (0.01)	0.13 (0.02)	0.14 (0.02)

Table 1 Means (se) from the multivariate analyses showing the main predisposing factors associated with calving assistance and uterine infections. Raw data means are shown with their respective standard errors.

Additionally, lower daily dry matter intakes (P<0.01) and milk yields (P<0.05) during the first 100 days in milk (DIM) were associated with higher incidence levels of uterine infection. Also, a higher incidence level of uterine infection was related to lower milk protein composition from one to 21 DIM (P<0.05) and a poorer body condition from 101 to 300 DIM (P<0.001). Cows with poorer drying off body condition scores and longer lactation lengths were associated with a higher incidence level of uterine infection (P<0.001 respectively).

Conclusion The predisposing factors influencing the occurrence of calving assistance can be influenced by suitable management to reduce the chances of oversized calves, the detrimental effects of twin births (such as calving assistance and retained placentas) and prenatal knowledge of the calf's sex. The occurrence of uterine infection can be reduced by minimising the need for assistance at calving.

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Effect of twinning on the feed intake, performance and reproductive health of dairy cows

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Introduction Twinning in dairy cows is looked upon as undesirable, due to management issues and resulting health problems. Continued increases in milk production over the last two decades in dairy herds has been associated with an increase in twinning rate (Wiltbank *et al.* 2000). The lactation number and level of milk production of the cow appear to be positively associated with increased ovulation rate and twinning (Wiltbank *et al.* 2000). Unfortunately, twinning is difficult to control by selection due to its association with milk yield and its low heritability (Komisarek and Dorynek, 2002). The aim of this study was to assess the effect of bearing twins on the health and performance of dairy cows.

Materials and methods Data from twin-bearing Holstein Friesian cows at the Langhill Dairy Research Centre between January 1990 and July 2002 were compared to single-bearing cows to determine if there was any significant difference in milk production, feed intakes, health and fertility. The Langhill herd are on a long-term genetic breeding and feeding systems project. Approximately equal numbers of select line (selected for kilograms of fat plus protein) and control line cows (average genetic merit for fat plus protein production) were offered either a high or low concentrate diet as a total mixed ration (TMR). There were 122 twin births (from 104 different cows) in the Langhill herd, a rate of 4.7 per cent during the study period. The periods before parturition (prepartum), at parturition and after parturition were studied (postpartum). A sample group of 71 twin calving cows could be paired to a single-bearing cow on calving date (during the same month) and exactly on feeding system, genetic line and parity. Of the 71 cows, 59 cows could also be paired to a single-bearing cow prior to parturition. Cows classified with metritis were cows diagnosed with a uterine infection 21 or less days post calving and those after 21 days were classified as suffering from chronic endometritis. The production and fertility data were analysed using REML in Genstat Version 7.2 (Lawes Agricultural Trust, 2004), with significance attributed at $P < 0.05$. The difference in twinning rate between genetic lines and subsequent reasons for culling and health problems was studied by calculating an odds ratio.

Results The incidence of twinning was higher in high genetic merit cows (select genetic line cows) and increased with parity ($r = 0.870$, $P < 0.01$). Compared to a single-bearing cow, a twin pregnancy was associated with a shorter gestation period ($P < 0.001$), an increased chance of a retained placenta after calving ($P < 0.01$) and a poorer body condition score post-calving ($P < 0.001$). The post-calving condition score of twin-bearing cows was negatively correlated with the total weight of calves born ($r = -0.235$, $P < 0.05$). Twin calving cows also had significantly more dystocia ($P < 0.05$), a higher chance of having a dead calf ($P < 0.001$), an increased number of days from calving to their first observed heat ($P < 0.001$) and number of days from calving to their first service ($P < 0.01$). Dystocia was positively correlated with the number of dead calves at birth ($r = 0.309$, $P < 0.01$) and with the total weight of calves born ($r = 0.211$, $P < 0.05$). Table 1 shows that twin-bearing cows on a low concentrate diet had an increased chance of having metritis and/or endometritis compared to single-bearing cows on the same diet. There was no significant difference in the reason for culling or the time at which cows were culled post-calving, however, twin-bearing cows on a low concentrate diet were 3.2 times more likely to be culled than a single-bearing cow (95% confidence interval = 0.17 to 0.61), with cows on a high concentrate diet having equally high levels of culling. There were no significant differences between twin and single producing cows' mean daily feed intakes, daily milk yields, total milk yields and milk fat and protein compositions during early, mid and late lactation pre and postpartum on a low or high concentrate diet.

Table 1 Main health problems for twin and single calving cows on a low concentrate or high concentrate diet. Percentage of cows with an incidence recorded.

Health Problem	Low concentrate diet				High concentrate diet			
	Twin	Single	Odds ratio	95% Confidence interval	Twin	Single	Odds ratio	95% Confidence interval
Metritis	22.6	3.2	2.27	0.03-0.35	17.5	7.5	1.35	-0.04-0.24
Endometritis	16.1	0	2.33	0.03-0.29	10	2.5	1.39	-0.03-0.18
Retained Placenta	25.8	0	3.03	0.10-0.41	27.5	0	3.57	0.14-0.41

Conclusion The body condition and drying off period of twin pregnant cows should be managed to avoid post-calving health and performance problems. Twinning can have a detrimental effect on the health and fertility of a dairy cow, however, it is recommended that by appropriate management of the body condition of the cow through nutrition, these adverse effects can be minimised.

Acknowledgements We are grateful to the farm staff and Ross McGinn for capturing the data.

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Reasons for exiting in Iranian Holstein cows

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Introduction Exiting is a complex issue, and many factors are involved in the decision to exit a cow. Farmer consider many cow factors, such as age, stage of lactation, milk production, health status, disposition, and reproductive performance, when determining whether or not a cow should be exited (Beaudeau *et al.*, 2000). The exiting decision may also be affected by economic factors, such as milk price, the price of exited cows, and the price and availability of replacement heifers. In addition, the attitudes of farmers have an effect on which cows are removed from a herd (Bascom and Young, 1998). Iranian dairy production has undergone significant and considerable structural changes during the last two decades with creation of larger herds. Replacement of dairy cows is a major expense for producers. To keep a cow until a higher parity allows the farmer to spread over a longer period the difference between the production cost of the replacement heifer and the carcass value of the exited cow (Seegers *et al.*, 1998). In Iran, the increased price for replacement heifer in the last several years has increased the interest in increasing productive life and there by lowering exiting rates. Analyses of the reason of exiting are needed to predict herd performance and maximizing herd profit. The objective of this study was to study the reasons for exiting in Iranian Holstein cows.

Materials and methods The exiting data from six large dairy farms during 1999 until 2006 were used. All cows in the study were Holstein. Milking occurred 2 or 3 times daily in milking parlour. During the period, the median number of cows in the study herds was 600. Data on cow and herd identification, calving, and exiting reason along with the date were recorded. The dependent variable of interest was age in days. The age in days was defined as the number of days between birth and exiting. The data were analyzed using the statistical software package JMP (SAS Institute Inc., NC, USA). The model included herd, number of abortion during life, calf's birth weight in the last calving, parity at exiting, twinning in the last calving, calving class (eutocia, dystocia, stillbirth, and abortion), cumulative first 60 days milk production in the last lactation, exiting reason (including sold for dairy purposes, low milk production, feet and legs problems, reproductive problems, death, mastitis, disease, and udder problems), birth date, season of year when cow was exited, and also days open.

Results The result showed that herd, number of abortion during life, parity, exiting category, birth date, and also days open had significant impact on age in days for exited cows ($P < 0.05$). Age at exiting averaged 6 years and 354d. The median was 5 years and 179 d, and 25% and 75% quartiles were 3 years and 244 d, and 8 years and 175 d, respectively. There was variability in survival between herds ($P < 0.001$). In terms of voluntary exiting, the exiting policy was different among farmers. Age in days increased with number of abortion during cow's life ($P < 0.001$). Increased days open was associated with increase in age in days at exiting ($P < 0.001$). The cow's risk of being exited was impact by the exiting reason ($P < 0.05$; Figure 1). Parity at exiting averaged 3.185. The median was 3 and 25% and 75% quartiles were 1 and 5, respectively.

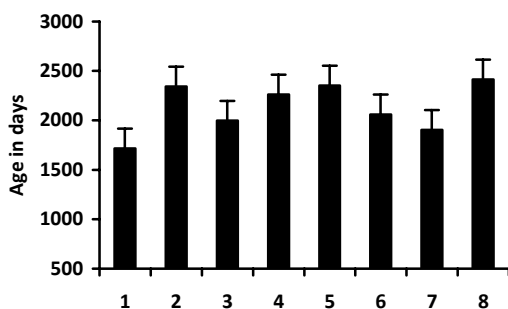


Figure 1 Effect of exiting reasons on age in days. (1= sold for dairy purposes, 2= low milk production, 3= feet and leg problems, 4= reproductive problems, 5= death, 6= mastitis, 7= diseases, 8= udder problem)

Conclusions The results of the present study demonstrate that sold for dairy purposes followed by diseases, and feet and leg problems were the main reasons to exit cows younger than the others. This research also indicates that dairy farmers consider many factors when deciding whether and when to exit a cow.

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Days in milk at exiting in Iranian Holstein dairy cows

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Introduction Exiting is the departure of cows from the herd for several reasons. Cows are exited for a variety of reasons. The predominant reasons for exiting are reproduction (i.e., failure to conceive), health, and low production (Bascom and Young, 1998). Half of the herd removals occur involuntarily and prematurely because of health disorders (Beaudeau *et al.*, 2000). On the other hand, the risk of exiting is not consistent across all stages of lactation. Cows experience the highest risk shortly after calving (Fetrow *et al.*, 2006). Cow longevity is highly related to dairy farm profit. Only a few previous studies have characterized exited cows based on the associations between exiting reasons and cow economic characteristic. One of this important characteristic is the lactation stage when the cow is exited. There is, therefore, a need to evaluate the overall exiting reasons and the stage of lactation the cows exited the herd. The objective of this study was to study the days in milk at exiting in Iranian Holstein dairy cows.

Materials and methods The exiting data from five large dairy farms during 1999 until 2006 were used. During the period the median number of cows in the study herds was 500. Data on cow and herd identification, calving and exiting dates were recorded. The exiting reasons were sold for dairy purposes, low milk production, feet and legs problems, reproductive problems, death, mastitis, disease, and udder problems. The dependent variable of interest was the number of days between calving and exiting. The data on the days in milk (DIM) when a cow was exited for the defined category were analyzed using general linear models. The means were separated using Tukey multiple range test. The distribution analyze for when a cow was exited during the lactation period was done using the statistical software package JMP (SAS Institute Inc., NC, USA).

Results Figure 1 shows DIM at exiting for different exiting category. Calving-to-exiting was impacted by exiting category ($P < 0.0001$). Calving-to-exiting intervals (mean \pm SE) were 107 ± 13 , 117 ± 11 , 158 ± 4 , 161 ± 12 , 202 ± 10 , 230 ± 33 , 242 ± 9 , and 379 ± 5 d for udder problem, mastitis, diseases, feet and leg problems, sold for dairy purposes, death, low milk production, and reproductive problems, respectively. Days in milk when cows were exited due to udder problems and mastitis were lower compare with the other categories. These results are in agreement with another study on reasons for exiting in French cows (Seegers *et al.*, 1998). In general, DIM at exiting averaged 217.20 d with standard error of mean 2.66 for the all exited cows (Figure 2). The median was 183 d and 25% and 75% quartiles were 41.75 and 350 d, respectively. Its distribution showed a progressive decrease in frequency.

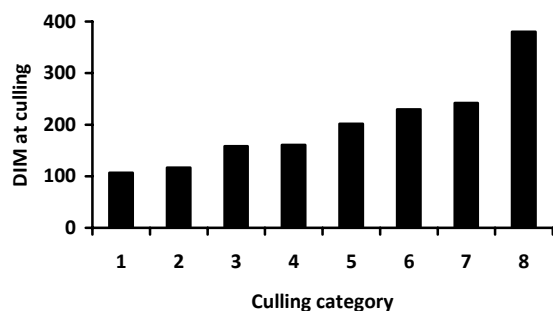


Figure 1 DIM at exiting for different exiting category. (1= udder problem, 2= mastitis, 3= diseases, 4= feet and leg problems, 5= sold for dairy purposes, 6= death, 7= low milk production, 8= reproductive problems)

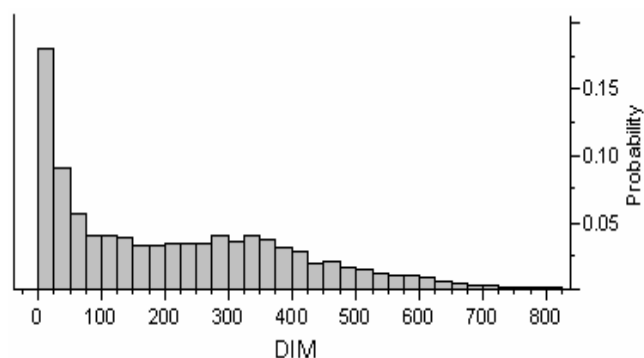


Figure 2 DIM at exiting

Conclusions The result of this study demonstrated that cows were exited differently according to the exiting category. Udder problem and reproductive problems had the lowest and greatest calving-to-exiting interval. The median time when a cow was exited suggested that it was preferred to exit a cow far after milk peak.

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Effects of pectin on production and urinary nitrogen excretion in lactating Saanen dairy goats

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Introduction Urinary nitrogen (N) is rapidly converted to ammonia (NH₃) during manure collection and storage, whereas faecal N is converted to NH₃ at a much slower rate. Developing nutritional strategies to shift N excretion from urine to faeces may reduce NH₃ from dairy manure which is implicated in reduced air quality. Previous work suggests that increasing post-ruminal fermentation may reduce urinary N and consequently manure NH₃ losses (Gressley and Armentano, 2005). Pectin is a class of neutral detergent-soluble fibre (NDSF) and studies in monogastrics have shown that it is completely degraded by resident intestinal bacteria (Buchanan *et al.*, 1994). Gut bacteria are capable of converting blood urea-N into NH₃ and subsequently microbial protein. Therefore, growth of intestinal microorganisms using energy supplied by pectin can result in a net conversion of blood urea N into faecal microbial N, thereby reducing urinary N. The aim was to determine the effect of increasing carbohydrate fermentation in the large intestine on urinary N in lactating dairy goats.

Material and methods Four primiparous lactating Saanen dairy goats (average body weight (BW) 41±1.5 kg, days in milk 48±2.1 and milk production 1.93±0.35 kg) were used in a 4*4 Latin squares design with 14-d periods. Goats were abomasal cannulated and housed in metabolism crates with free access to water. Goats were fed with a basal diet (40% lucerne hay and 60% concentrates on dry matter (DM) basis) and offered at 95% of its estimated ad libitum intake throughout the experiment. Treatments were abomasal infusion of saline only (0 Pectin), saline containing 40, 80 or 120 g/day pectin. High-methoxyl slow-set citrus pectin was infused at 2.4 l/d from day 6 to 14 of each period. Total faecal and urine output was collected during the last 4 days of each period in order to calculate N balance. Faecal and urine samples were collected twice daily during the last 4 days of each period and prior to N analysis the samples were bulked within goat and period, with an equal portion taken from each sampling time. Duplicate milk samples from both a.m. and p.m. milking were taken during the final 4 days of each period and analysed for milk constituents. Data were analysed using a general linear model (GLM) in SAS (1999) and the significance of any infusion effects were detected using the Duncan's multiple range tests.

Results Dry matter intake decreased from 2250 g/day for 0 g/d to 1985 g/day for 120 g/d pectin infusion. Infusion of 120 g/d also decreased milk yield and 4% fat corrected milk (FCM) yield. Other treatments did not affect milk production. Treatments had no effect on milk composition. Increasing abomasal pectin infusion decrease urinary N and increase faecal N. Urinary N decreased from 3.92 and 4.32% with 80 and 120 g/d pectin infusion respectively.

Table 1 Effect of abomasal pectin infusion on intake, milk production and composition

	Pectin (g/day)				S.E.M.
	0	40	80	120	
Basal DM eaten ¹	2250 ^a	2215 ^a	2190 ^{ab}	1985 ^b	79.4
Total DM input ²	2250 ^a	2245 ^a	2270 ^a	2105 ^b	89.42
Milk yeild (Kg/day)	1.95 ^a	1.90 ^a	1.90 ^a	1.75 ^b	0.09
4% FCM yeild (Kg/day)	1.77 ^a	1.76 ^a	1.77 ^a	1.60 ^a	0.08
Protein (%)	3.01	3.10	3.05	3.01	0.06
Fat (%)	3.40	3.55	3.50	3.43	0.10
Lactose (%)	4.63	4.65	4.60	4.62	0.03
total solid (%)	11.65	11.90	11.76	11.67	0.70
solid not fat (%)	8.25	8.34	8.26	8.24	0.58

¹ Intake of the basal ration only, not including pectin infused; ² Intake of the basal ration plus pectin infused; S.E.M, standard error of the mean

Table 2 Effect of abomasal pectin infusion on intake and excretion of nitrogen

	Pectin (g/day)				S.E.M.
	0	40	80	120	
Milk, % of intake	15.60	15.85	15.76	15.56	0.65
Faeces, % of intake	25.2 ^a	26.5 ^a	28.8 ^b	29.4 ^b	0.41
Urine, % of intake	36.31 ^a	34.79 ^a	32.39 ^b	31.97 ^b	0.52
Urine N/(faecal N + urine N)	59.20 ^a	57.25 ^a	53.69 ^b	52.42 ^b	0.63
Retained N, (% of intake)	23.05	23.21	23.80	23.34	0.91

Nitrogen retained = intake N - milk N - urine N - faecal N.

Conclusions Although similar studies had not been performed in lactating dairy goats, these data support the hypothesis that increasing post-ruminal fermentation in dairy goats might shift some N excretion from urine to faeces. Infusion of 120 g/d pectin negatively affects production but 80 g/d abomasal fibre shifted approximately 3.9% from urine to faeces without adversely affect production.

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Digestibility of low and high-roughage diets by two breeds of sheep

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Introduction It is a general believe that the local sheep breed Churra da Terra Quente (CTQ), reared in the Northeast of Portugal is well adapted to the conditions of its production system. However, there are large gaps on our knowledge to allow understanding of such claimed adaptation. Several researchers have found differences in digestibility between sheep breeds (*e.g.* Givens and Moss, 1994; López *et al.*, 2001), suggesting that those fed natural resources can have an improved ability to digest fibre. This experiment was designed to compare digestibility coefficients of a high- and a low-roughage diet measured on sheep from CTQ and Ile-de-France (IF) breeds at the same degree of maturity.

Materials and methods Ten female lambs of each breed (30.5 ± 0.7 and 50.3 ± 1.0 kg, corresponding to 0.66 of estimated mature body weight of CTQ and IF respectively) were randomly assigned to one of two dietary treatments, according to a 2x2 factorial design: low- and high-energy diets (LE and HE, respectively; Table 1). The concentrates were pelleted and the hay was chopped coarsely (4 cm in length) to reduce selection and wastage. Animals were housed in metabolism cages in a room with controlled temperature and humidity and given free access to water. The digestibility trial comprised a first period of 14-d for adaptation and a 7-d period for total faeces collection. The concentrates were offered once a day at 0900 prior to the hay and the hay during the day with allowance of 20–25% refusals. Samples of feeds, hay refused and faeces were taken daily for dry matter (DM) determination, bulked for the entire experiment and analyzed for ash (AOAC 1990), neutral detergent fibre (NDF) as described by van Soest *et al.* (1991) and gross energy (GE) using an adiabatic bomb calorimeter (Parr, model 1241). Data were analysed by ANOVA and means compared using the least significant difference ($P < 0.05$).

Table 1 Chemical composition (% of DM) and estimated ME and ERDP contents of the experimental feeds and diets.

Item	Hay (H)	Concentrate LE (CLE)	Concentrate HE (CHE)	Diet LE (75H:25CLE)	Diet HE (30H:70CHE)
Chemical composition					
OM	93.6	91.5	88.7	93.1	90.2
NDF	68.5	25.3	28.5	57.7	40.5
CP	7.1	34.1	21.1	13.9	16.9
ME (MJ/ kg DM) ^a	7.6	13.2	13.2	9.0	11.5
ERDP (g/kg DM) ^a	35.0	199.5	123.7	76.1	97.1
ERDP:FME (g/MJ) ^a	-	-	-	9.7	9.9

^a Calculated from tabular values (AFRC, 1993); ERDP: effective rumen degradable protein; FME: fermentable ME.

Results Intake of DM was similar in both breeds (Table 2), a higher intake being observed for diet HE ($P < 0.001$). As anticipated, the digestibility coefficients were higher for diet HE ($P < 0.001$). The digestibility of organic matter (OM) measured in IF animals were 2.7 and 0.8 percent units higher for diets LE and HE, respectively, but breeds did not differ in NDF or energy digestibility. No breed x diet interaction was observed for any of the measurements carried out.

Table 2 Effect of breed and diet on DM intake (DMI) and digestibility coefficients.

Item	CTQ		IF		SEM	Breed		Diet		P-value	
	LE	HE	LE	HE		CTQ	IF	LE	HE	Breed	Diet
DMI (g/kg BW ^{0.75})	73.4	30.2	74.5	30.4	0.774	51.8	52.4	73.9	30.3	0.424	<0.001
Digestibility (%)											
OM	58.5	69.5	61.2	70.3	0.855	64.0	65.7	59.7	69.8	0.048	<0.001
NDF	51.7	58.2	52.5	59.6	1.089	54.9	56.1	52.1	58.8	0.293	<0.001
GE	60.6	73.1	62.2	73.7	0.833	66.9	68.0	61.3	73.4	0.175	<0.001

Conclusions The differences in digestibility coefficients between breeds detected in the current experiment are small, and they do not support the hypothesis that the breed evolved in areas where diets are based on low quality pastures had a higher capacity to digest fibre. So, research on other aspects of digestive and metabolic processes is needed to understand the adaptation of CTQ to its production system.

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Effects of condensed tannins from *Acacia mearnsii* on *Trichostrongylus colubriformis* in experimentally infected Brazilian sheep

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Introduction The over-dependency and misuse of the chemotherapeutic drugs available to parasite control with consequent development of anthelmintic resistance, mainly in small ruminants, has been demanded alternative sources to control gastrointestinal nematodes in sheep. The aim of the present study was to investigate the potential anthelmintic effects of condensed tannins (CT) from acacia extract (AE) on *Trichostrongylus colubriformis* in experimentally infected sheep.

Materials and methods Eighteen four-month-old worm-free Santa Inês lambs were divided in three groups and housed indoors-feeding hay and concentrate (diet with 13% of crude protein). Twelve animals were experimentally infected with 20,000 L3 of *T. colubriformis* and six were kept as non-infected control. Group I (GI) was the non-infected control; group II (GII) was infected control and group III (GIII) was supplied with condensed tannin (CT) source. The used source was the acacia extract (AE) from *Acacia mearnsii*, which contained 15% of CT. Thirty days post-infection (p.i.), Group III was supplemented with AE (1.6 g/kg of body weight) per day added to concentrate, during 10 days. The same amount (1.6 g/kg of B.W.) of dry molasses powder (DMP) was mixture with the AE to improve the diet palatability. Animals from Group II were supplemented only with DMP in the same dose of GIII. Faecal eggs counts (FEC) were carried out weekly until day 21 p.i., twice a week from day 21 to 30 p.i. and each two days during and after the treatment with CT (from day 30 to 45 p.i.). During the week of evaluation of AE, five coprocultures and egg viability tests were carried out for each infected group (days: 0, 4, 7, 10 and 14 of CT treatments) and the number of infective larvae (L3) per gram of faeces (LPG) and number of L1 reached from eggs (egg viability percentage) was determined. The values of FEC, LPG, egg viability, number of FEC accumulated per animal during the treatment period, number of viable eggs released, and worm burden were analyzed using the general linear model (GLM) with repeated measurements procedure of SAS. The means were compared by Duncan test and the differences between means with $P < 0.05$ were considered statistically different.

Results On day 32 p.i. (second day of treatment with AE) a significant reduction on FEC ($P < 0.05$) was detected between GIII and GII. However, analyzing the average of total accumulated amount of FEC released per group, during the 10 days of the treatment, a significant reduction in CT treated lambs ($P < 0.05$) was detected, with FEC averages, per animal, during this period, of 11500 and 5317, respectively for GII and GIII. The values of egg viability, analyzed on days 0, 4, 7, 10 and 14 post-treatment (p.t.), were statistically reduced ($P < 0.05$) on days 4, 7 and 10 p.t. (Table 1). In the same way, values of viable eggs (FEC values x egg viability %) per group were lower in CT treated group ($P < 0.05$) on days 4 and 10, with tendency ($P = 0.06$) of reduction on day 7. The number of L3 recovered per gram of faeces (LPG) was lower ($P < 0.05$), at the days 10 and 14 p.t., in Group III (Table 1). The use of CT on diet caused no significant difference on body-weight and worm burden ($P > 0.05$). Animals from GI have not presented nematode eggs on faeces.

Table 1 Number of third stage larvae per gram of faeces (LPG) and egg viability (L1%) of *Trichostrongylus colubriformis*. In faeces samples collected from lambs from infected control groups (GII) and group treated with acacia extract during 10 days (GIII).

Groups	Days Post-Treatment									
	0		4		7		10		14	
	LPG	L1 %	LPG	L1 %	LPG	L1 %	LPG	L1 %	LPG	L1 %
GII	5790 ^a	71 ^a	15690 ^a	90,5 ^a	5340 ^a	71,5 ^a	1170 ^a	31,5 ^a	47400 ^a	98,5 ^a
GIII	11910 ^a	70 ^a	3900 ^a	9,5 ^b	1830 ^a	54,5 ^b	0 ^b	3 ^b	900 ^b	59,50 ^a

Different letter on the same column – ($P < 0.05$)

Conclusions Probably, the anthelmintic effect is not only originated by tannins and others secondary plants metabolites could be involved. However, the CT uses in nematode control cannot be discarded, not even their use, at least in alternative and prophylactic controls, reducing the pasture contamination, nematode reinfection in sheep and the number of chemical treatments.

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The effect of dietary intake of phosphorus on true absorption and excretion of phosphorus in Brazilian Santa Ines sheep

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Introduction Phosphorus (P) is an essential nutrient in livestock production, involved in most metabolic processes in the body, and with an impact on bone development and growth. Diet supplementation with P can improve productivity, but at excess levels the P utilization can be low, and the high P excretion will lead to environmental pollution (Tamminga, 2003). P supplementation also adds substantially to the cost of feed. Using the isotopic dilution technique (Vitti *et al.*, 2000), we have studied the effect of different P levels in diet on P absorption and excretion in Brazilian sheep, and identified the level at which true P absorption saturates.

Material and methods Twenty four Santa Ines (Brazilian breed) male sheep were used in a random design. Treatments were a basal diet (T0 - 1.47 g P / animal / day) and three P supplemented feeds (T2 - 3.54 g P / animal / day, T4 - 5.70 g P / animal / day, T6 - 7.48 g P / animal / day); the P supplement was dicalcium phosphate. The basal diet was composed of cassava meal, soybean meal, urea, mineral mixture and hay (Tifton 85). Before assay, all animals were treated against parasites and distributed into stalls (six animals per treatment). After 21 days of adaptation the animals were weighted and allocated to individual metabolic cages. After a further 5 days of adaptation, each animal was intravenously injected with 7.7 MBq of ³²P. Samples of blood, urine, and faeces were collected every 24 h for 7 days following the injection. Total inorganic P and ³²P in the plasma, urine and faeces were determined, from which the endogenous P and true P absorption were estimated (Vitti *et al.*, 2000). Feed P level effects were tested by ANOVA and means compared by the Tukey test.

Table 1 Effect of different levels of P on P metabolism in sheep

Parameter (mean over 6 animals / diet)	Diet			
	T0	T2	T4	T6
Live weight (kg)	33.92 ^a	33.92 ^a	33.33 ^a	33.50 ^a
P intake (mg/kg LW/day)	43.24 ^d	104.37 ^c	170.87 ^b	223.25 ^a
P plasma (mg/dl)	6.66 ^a	7.91 ^a	8.78 ^a	9.02 ^a
P in faeces (mg/kg LW/day)	32.36 ^d	94.04 ^c	132.61 ^b	195.61 ^a
P urine (mg/kg LW/day)	0.10 ^a	0.31 ^a	0.66 ^a	1.69 ^a
Endogenous P in faeces (mg/kg LW/day)	17.58 ^c	47.05 ^b	71.99 ^a	72.55 ^a
True P absorption (mg/kg LW/day)	28.46 ^c	57.37 ^b	100.18 ^a	105.13 ^a
P retention (mg/kg LW/day)	10.78 ^b	13.28 ^b	44.19 ^a	23.52 ^{ab}

^{a, b, c, d} within rows, denotes significantly different ($p < 0.05$)

^{ab} not significantly different ($p < 0.05$) from ^a or ^b.

Results According to the standard classification scheme (NRC, 2006), the intake of P (Table 1) was low (T0), adequate (T2 and T4), and in excess (T6). The added P has no statistically significant ($p < 0.05$) effect on both P in plasma (normal range 4 - 9 mg/dl), and P in urine (0.23 % - 0.76 % P intake). There is a significant increase for P in faeces with P intake across all treatments, rising to a maximum of 88 % P intake for T6. The ³²P dilution results showed that while the endogenous P in faeces and true P absorption increased with P intake for T0 to T4, there was no further increase between T4 and T6. True P absorption and P retention saturate between T4 and T6.

Conclusions Supplementing the basal diet with P to give feed with a P level of up to 5.70 g P / animal / day leads to a progressive increase in the true P absorption from 28.46 mg/kg LW/day (1.47 g P / animal / day) to 100.18 mg/kg LW/day (5.70 g P / animal / day), which is beneficial to productivity. A further increase in the P supplementation to 7.48 g P / animal / day leads to no increase in the true P absorption, but there is an increase in the P in faeces. 7.48 g P / animal / day represents a clear excess - no productivity benefits, but with increased P contamination from P in faeces.

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Seasonal wool growth and staple strength on Baluchi sheep breed

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Introduction Seasonal wool production which is under some genetic performance appears to be largely responsible for variation in staple strength, fibre diameter and its confidence of variation (Butler, 1994). Baluchi sheep breed live in central and eastern parts of Iran under different altitude and weather with diverse conditions. This breed comprises 19 per cent of Iranian sheep population and its fibres known as a good quality of carpet wool. This study aimed to evaluate the seasonal effects on native wool which improving wool traits by nutritional strategy on adverse time.

Materials and methods The fifty – two Baluchi sheep of one year age from Abas Abad station located in Northeast of Iran were allowed to this experiment for a period of two years. The sheep were grazing on pasture and beside also used a maintenance ration. Periodic wool growth, fibre diameter and staple strength were measured from mid side patches taken every six month. (1) The projection microscope technique was used in accordance with ASTM D2130-78 to determine fibre diameter and Almeter instrument for fibre length. In order to determine the staple strength, the clamped fibre tuft were placed in tensile testing machine (Instron co.) and used 1N capacity load cell with the pulling clamps moved at rate of 25cm/mm (ASTM.D-1294) and the staple lengths were determined by ruler. Data were analyzed by repeated measurement and general liner model (GLM) using SAS software package.

Results The result indicated that fleece weight were significantly ($P<0.001$) affected by season. The wool growth was greatest during autumn and winter and lowest during sprig and summer ($P<0.001$). Staple lengths from shoulder, flank and rump areas and fibre diameter were significantly ($P<0.001$) different but versus the fleece weight during autumn - winter were lowest than other seasons. Staple length at rump area was longer than those from shoulder and flank regions. But of course, a significant effect of time of wool harvest on coefficient of variability of mean fibre diameter and tenacity were determined.

Table 1 Least squares means and standard errors for spring and autumn wool characteristics of Baluchi sheep

Character	Fleece weight(kg)	staple length(cm)			Length(mm)		diameter (μ)	Cv diameter (%)	tenacity (gf/tex)
		shoulder	flank	rump	Hauteur	Barbe			
Autumn & Winter Wool Growth	1.32 ^a (0.04)	5.92 ^b (0.17)	5.92 ^a (0.17)	6.87 ^b (0.17)	22.3 ^b (0.8)	36.6 ^b (1.17)	27.36 ^b (0.54)	41.9 ^a (1.21)	4.88 ^a (0.27)
Spring & Summer Wool Growth	0.91 ^b (0.04)	6.57 ^a (0.17)	5.62 ^b (0.17)	7.42 ^a (0.17)	28.8 ^a (0.9)	50.8 ^a (1.2)	28.98 ^a (0.55)	38.5 ^b (1.24)	3.52 ^b (0.27)

*. a, b, c: Means with different superscripts in the same column differ significantly ($P<0.001$).

*: Hauteur and Barbe represent the length of fibres that calculated from the proportion by titre and mass of fibres in silver

Conclusion A big variation was observed in fibre diameter (19.6 – 39 μ) which, such variation can not be attributed to seasonal variation. The first spring wool were coarser (7 – 9 μ) than the other season, it is probably due to more available feed in range lands which better supported yearling ewes also because didn't effect of pregnancy and lambing in this old. More study is needed to investigate factors influencing seasonal growth of wool and staple soundness and find simply attribute to avoid stress events to result in decrease variation in wool performance. Studies shown, lowest wool production tends to occur in autumn and winter in almost all sheep that rearing in tropics regions (Bellitti and Vonghia, 1973), but the result of this study determined adverse effect that lowest wool growth tends to occur during spring– summer season.

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Effect of a mannan oligosaccharide supplement (Bio-Mos®) on the growth of indoor-reared lambs from birth until weaning on a commercial farm

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Introduction Early finished lambs are able to command a higher premium at slaughter. Improvement in growth rate can increase the profitability of the enterprise. The use of yeast cell wall preparations, in particular the mannan oligosaccharide portion, has been shown to improve intestinal tract health and thus growth rates in other species (Uni & Smirnov, 2006; Rosen, 2006). Farm studies have suggested that the inclusion of mannan oligosaccharides have the greatest effect in young animals prior to weaning. The aim of the current study was to determine the effect of feeding a mannan oligosaccharide on the growth rates of early finished lambs reared indoors on a commercial farm from birth until weaning.

Materials and methods Prior to lambing, 380 ewes (Finn-Dorset x Suffolk or Charollais) were randomly allocated to one of two treatments, to provide approximately 350 lambs per treatment with an equal split of single, twin and triplet lambs per treatment. At birth (5-16 December 2006), the lambs were weighed, tagged and, 24 hours later, assigned, with their dam, to one side of a poly-tunnel. The poly tunnel contained straw bedding on either side of a central passageway that ran the length of the tunnel with open access of this passage at both ends. Ewes were fed a total mixed ration of grass silage, soybean meal and minerals *ad libitum*. From 2 days of age, lambs were offered one of two commercial creep feeds (Countrywide Farmers Ltd). One of the creep feeds was supplemented with 2kg per tonne mannan oligosaccharide (Bio-Mos®, Alltech Inc, USA) whereas the other was unsupplemented (Control). Lambs were again weighed at weaning at approximately 10 weeks of age. Incidence of disease in each group and mortality was recorded. Intakes of creep feed was recorded. Data was analysed by ANOVA using UNISTAT 5.6 (UNISTAT Ltd, London, UK).

Results There was no significant difference in birth weight of the lambs between groups. Lambs that received Bio-Mos® in the creep feed were significantly heavier (kg) at weaning ($P < 0.001$), had a greater total weight gain (kg) during this period ($P < 0.001$) and daily weight gain (g/day) was also greater ($P = 0.001$) compared with the control lambs. Numerically, the lambs in the Bio-Mos® group consumed a greater amount of the creep feed over the entire trial period but the overall estimated feed conversion ratio was no different between groups. Although there could be a confounding effect from the side of the poly tunnel to which the groups were allocated, the lambs in the Bio-Mos® group had numerically fewer treatments for pneumonia-type symptoms and mortality due to disease was also numerically lower. There was no difference in the incidence of other diseases between groups.

Table 1 Effect of mannan oligosaccharide supplementation on weight and weight gain in indoor-reared early lambs

	Units	Control	Bio-Mos®	s.e.d.	P-value
Birth Weight	kg	4.26	4.32	0.073	0.429
Weaning Weight	kg	19.38	20.75	0.329	<0.001
Total Weight Gain	kg	15.12	16.43	0.297	<0.001
Daily Gain	g/day	227	243	0.004	0.001
N		338	336		

Table 2 Feed use, Estimated FCR, disease treatments and mortality

	Units	Control	Bio-Mos®
Total Feed Intake	kg/lamb	17.94	19.49
FCR	kg feed/kg gain	1.19	1.19
Disease Treatments		17	7
Mortality		9	2
N		338	336

Conclusions The addition of 2kg mannan oligosaccharide (Bio-Mos®) per tonne increased weaning weight and total weight gain from birth to weaning ($P < 0.001$) and increased daily liveweight gain ($P = 0.001$) in lambs born in early December and reared indoors in poly tunnels. With faster weights gains through to weaning, the use of this mannan oligosaccharide may allow for lambs to be weaned earlier and finished sooner, gaining a premium in the market place. Mannan oligosaccharide (Bio-Mos®) could be used in lamb creep feed to improve growth rates in early reared lambs without affecting health.

Acknowledgements Financial and product support from Countrywide Farmers and Alltech (UK) Ltd are gratefully acknowledged.

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Effects of plane of nutrition of ewes in early and mid pregnancy on performance of the offspring: female reproduction and male carcass characteristics

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Introduction Alterations of foetal development *in utero* can affect the structure and physiology of the adult offspring, a process known as “foetal programming” (Barker, 1994). Results from the first phase of this study indicated that offspring from mature ewes offered a restricted energy intake in early pregnancy were heavier at birth and had improved survival rates to weaning (Muñoz *et al.*, 2006). The aim of the second phase of the study was to determine the effects of plane of nutrition in early and mid pregnancy on carcass characteristics of male offspring and on reproductive performance and concentrations of the adiposity indicator, leptin, in female offspring, post-weaning.

Material and methods Between days 0 and 39 after synchronized mating (early pregnancy, EP) multiparous ewes were allowed 60% (low, L-EP), 100% (medium, M-EP) or 200% (high, H-EP) of requirements for maintenance (M). Between days 40 and 90 (mid pregnancy, MP), ewes were allowed either 80% (M-MP) or 140% (H-MP) of their M requirement. Between days -14 and 90 post-mating, ewes received selenium (Se) treatments providing either no Se (control) or Se supplement (1 g of Selplex® = intake of 0.5 mg Se/ewe/day). After day 90 of gestation, all ewes were fed to meet requirements for late pregnancy. Male offspring (n = 73) were reared on a grass-based system and slaughtered at 42, 46 or 50 kg live weight (LW). Carcasses were commercially graded for conformation (EUROP classification) and fat classification (1 = lean, 5 = high fat cover). Kidney and pelvic fat were weighed and subcutaneous fat depth was measured over the *l. dorsi*. Female offspring (n = 46) were reared on a grass-based system and mated at 8 months of age. Subsequent performance was recorded until weaning of their first lamb crop. Monthly jugular blood samples were collected throughout gestation for determination of plasma leptin concentrations by double antibody radioimmunoassay (RIA) using a guinea-pig anti-ovine leptin antiserum. Recorded data included LW and BCS at monthly intervals, litter size, gender of lamb and LW at birth, 6 and 16 weeks of age (weaning). Statistical analysis was performed as a factorial design with nutrition in early and mid pregnancy and Se supplementation as main effects and adjusting for dam and sire breed, siblings and field. The male model was additionally adjusted for slaughter weight (LWS) and for fat classification (FC) in alternative analyses.

Results There were no interactions between plane of nutrition in pregnancy and Se supplementation. Only the effects of plane of nutrition are reported in this paper. From weaning onwards, female offspring of dams offered either diet L-EP followed by H-MP, or diet H-EP followed by M-MP were heavier than the other females. For example 6w post-lambing, LW of females were L-EP/M-MP=48.3, L-EP/H-MP=59.6, M-EP/M-MP=51.9, M-EP/H-MP=52.4, H-EP/M-MP=54.3 and H-EP/H-MP=48.7 kg, sed 5.73, P<0.01). Leptin concentrations of female offspring varied with plane of nutrition in early pregnancy (Figure 1). Reproductive performance of female offspring was not affected by treatments (P>0.05). H-EP female offspring gave birth to heavier lambs than M- and L-EP offspring (4.3, 3.3 and 3.4 kg, respectively, sed 0.31, P<0.01), while M-MP offspring had lighter lambs than H-MP offspring (3.3 vs. 4.0 kg, sed 0.26, P<0.05). Male offspring of dams offered diet L-EP had poorer carcass conformation and increased fat depths over the loin (Table 1). Male offspring of ewes offered diet L-EP followed by H-MP, or diet H-EP followed by M-MP had increased adiposity as shown by kidney and pelvic fat amounts.

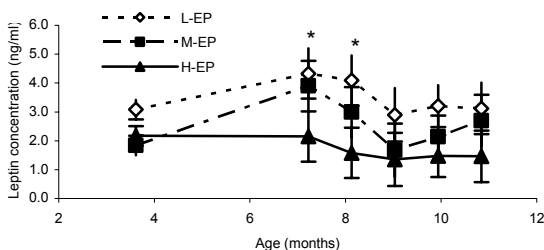


Figure 1 Effect of plane of nutrition in early pregnancy on female offspring leptin concentrations post-weaning. * Significant difference (P < 0.05) between diets L-EP and H-EP

Table 1 Effect of plane of nutrition in early and mid pregnancy on male offspring carcass characteristics

	Conformation classification		Fat depth over <i>l. dorsi</i> (mm)		Kidney and pelvic fat (g)	
	LWS	FC	LWS	FC	LWS	FC
L-EP / M-MP	3.0	3.1	2.1	2.1	197	190
L-EP / H-MP	3.3	3.3	2.6	2.3	302	277
M-EP / M-MP	3.6	3.6	1.6	1.8	211	239
M-EP / H-MP	3.6	3.6	1.9	2.0	265	267
H-EP / M-MP	3.2	3.4	1.7	2.0	327	361
H-EP / H-MP	3.2	3.2	1.6	1.7	212	204
s.e.d.	0.24	0.28	0.36	0.40	41.8	41.8
Significance						
EP	*	*	*			
MP						
EP X MP					**	***

Conclusions Changes undergone to compensate for nutrient snortage or excess in early and mid pregnancy can potentially alter offspring bodyweight, adiposity, conformation and leptin concentrations. Leptin results are especially interesting in the light of increasing evidence of associations between leptin and female fertility and leptin and immune competency.

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Effect of supplemented diet by sucrose or starch on ruminal pH and ammonia-N concentration in Holstein steers

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Introduction High producing dairy cows are often fed large quantities of high quality proteins, and because ruminal protein degradation is not directly coupled to microbial protein synthesis, ruminal NH₃-N production is always excessive and ultimately lost by urinary N excretion. Increasing the supply of ruminally fermentable carbohydrates can reduce ruminal ammonia-N concentration and increase milk protein yield (Sannes *et al.*, 2002). The addition of sugar has increased ruminal and whole animal N efficiency in sheep and steers, reportedly to a greater extent than starch (Chamberlain *et al.*, 1993). The objective of this work was to investigate the effect of diets containing different non fibre carbohydrates (NFC, sucrose or starch) on ruminal pH and ammonia-N concentration in Holstein steers.

Materials and methods Four fistulated Holstein steers (BW=280, SD 15kg) were assigned to a 4X4 Latin square with 21 day periods; 17 days diet adjustment and 4 days sample collection. The basal diet contained lucerne hay, barley grain, soybean meal and sugar beet pulp (400, 290, 190 and 50g/kg respectively). Starch (St) or sucrose (Su) or a 1:1 mixture of starch and sucrose (St+Su) was added to the basal diet at the rate of 70g/kg DM. Diets were offered at 2-2.5 times maintenance requirements (7kg DM/day). Animals were fed twice daily at 08:30 and 16:30H. Rumen liquids were sampled before and 0.5, 1, 2, 3, 4, 5, 6, 7, and 8hrs after the morning feed. Rumen pH was measured directly (691 pH meter) and rumen ammonia-N determined by steam distillation (Kjeltec Auto, 1300, Foss Electric, Copenhagen, Denmark). Data were analyzed using general linear model procedure of SAS (2003) with Duncan's test for the comparison of means (P<0.05). Statistical model was: $Y_{ijk} = \mu + T_i + C_j + P_k + \varepsilon_{ijk}$, where Y_{ijk} is dependent variable, μ is the overall mean, T_i is treatment effect, C_j is cow effect, P_k is period effect, and ε_{ijk} is error.

Results A significant effect of supplemental NFC on ruminal ammonia-N concentration was detected. However, no effect on ruminal pH was observed (Figure 1). Generally, ruminal ammonia-N concentration was higher when steers were fed the basal diet compared with St and Su (P<0.01).

Conclusions Results of the present experiment indicated that ammonia-N concentration was lower when steers were fed St or Su than only the basal diet. It has been proposed that when energy is a limiting factor in the rumen, micro-organisms degrade feed protein to ammonia and ammonia uptake by ruminal bacteria is inhibited (Nocek and Russell, 1988). A previous study demonstrated that water soluble sugars caused decreased ammonia concentration in the rumen, through decreased ammonia production, while starch caused increase in the uptake of ammonia for microbial protein synthesis (Hristov *et al.*, 2005). Results of the present study confirmed the finding of Chamberlain *et al.*, (1993) that addition of sugars decreased N losses. This positive effect of NFC on ruminal ammonia concentration decreased the metabolic effect of ammonia in host animal.

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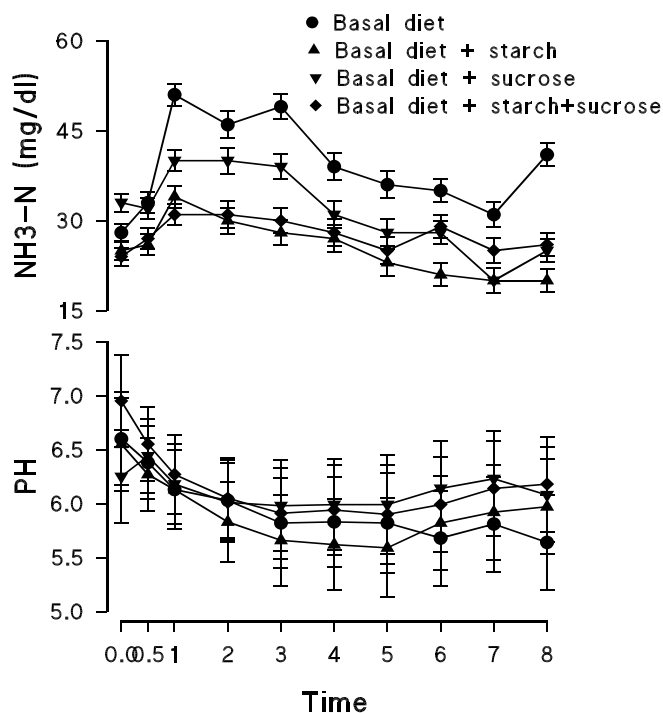


Figure 1 Hourly ruminal pH and ammonia-N concentration (mg/ dl) measured in steers fed starch or sucrose supplemented diets

Quantitative aspects of phosphorus metabolism in beef cattle using the isotopic dilution technique

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Introduction The availability of nutrients in low concentration in diets is difficult to be accessed by means of experiments like apparent digestibility assays. That is because the endogenous contribution underestimates the true availability. The best tool to study the true availability of minerals is the *in vivo* isotopic dilution technique. Phosphorus (P) and calcium (Ca) are the most studied minerals due to their great importance in animal nutrition. P is one of the most polluting nutrients because of high husbandry concentrations in restricted areas (Tamminga, 1992). There are some remaining doubts concerning the true digestibility of phosphorus. The present study compiles data from previous studies dealing with true availability of different P sources in beef cattle to estimate pollution potential of animal excretions.

Material and methods Database consisted of results from experiments carried out at the Centre for Nuclear Energy in Agriculture (CENA/USP, Piracicaba, Brazil), according to recommendations of the Radiological Protection Service and the Animal Welfare Committee. In those experiments, beef cattle (n=36) were fed different sources of P: dicalcium phosphate (n=11; LW=211±28); Tapira rock phosphate (n=11; LW=193±24); Patos de Minas rock phosphate (n=11; LW=196±33); and super triple phosphate (n=3; LW=211±7). True absorption of phosphorus was determined by isotopic dilution technique (Lofgreen, 1960). The animals were kept in metabolic cages for 21 days, designed for isotope studies, and a single dose was given to each animal, comprising 14.0 MBq of ³²P in 1 mL of sterile isotonic saline solution, into the right jugular vein. Blood samples were withdrawn from the left jugular vein at 24h intervals for 7 days. From the time of injection, P balance was recorded daily for 7 days through total collection of feed, refusals, faeces and urine. All parameters (P_{ING}: ingested P; P_{ABS}: absorbed P; P_{FECTOT}: total faecal P excretion; P_{FECENDO}: endogenous faecal P; and P_{FECNABS}: dietary non absorbed P) were normalized by live weight (LW), and are expressed as mg P/kg LW/day. Quadratic and linear regressions between P_{ING} and the other parameters were tested, using PROC REG of the SAS system (SAS, 2000).

Results Mean availabilities of P from tested sources were 0.659 (SD=0.082), 0.483 (SD=0.095), 0.465 (SD=0.069), and 0.576 (SD=0.039), respectively for dicalcium phosphate, Tapira rock phosphate, Patos de Minas rock phosphate, and super triple phosphate. P intake (P_{ING}) ranged from 41.4 to 73.5 mg/kg LW/d, P_{FECTOT} from 25.8 to 52.1 mg/kg LW/d, P_{FECENDO} from 6.5 to 22.2 mg/kg LW/d, P_{FECNABS} from 12.1 to 38.5 mg/kg LW/d, and P_{ABS} from 15.5 to 45.1 mg/kg LW/d. No significant quadratic effect was observed. Linear regressions are summarized in Table 1. No intercept value was significant. Therefore, considering just the slope, 0.62 of ingested P was excreted via faeces, in other words, the apparent availability of P was just 0.38. However, using the isotopic dilution technique, the mean true availability was 0.60.

Table 1 Linear regressions between P_{ING} (x) and other parameters (y) in beef cattle fed diets with four different sources of phosphorus

y	Slope			Intercept			R ² (c)	RMS E ^(d)
	value	s.e.m. (a)	P ^(b)	value	s.e.m. (a)	P ^(b)		
P _{FECTOT}	0.623	0.134	<0.0001	5.42	7.335	0.465	0.39	6.553
P _{FECNABS}	0.4	0.126	0.003	3.254	6.883	0.64	0.228	6.148
P _{FECENDO}	0.227	0.075	0.005	2.168	4.114	0.602	0.21	3.675
P _{ABS}	0.6	0.126	<0.0001	-3.253	6.883	0.637	0.4	6.148

(a) s.e.m.: standard error of means

(b) P: probability

(c) R²: regression determination coefficient

(d) RMSE: root MSE

Conclusion Based on those results, low available sources can be used in animal nutrition but the environmental damages must to be taken into account because P excretion can promote environmental pollution. Even common used P sources (like dicalcium phosphate) can generate the problem.

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Effect of rearing dairy-bred beef calves on once or twice per day milk feeding systems to weaning at 6 weeks old

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Introduction With the continued expansion of livestock units in the UK there is often an increased number of cattle reared per stockperson. Artificially reared calves are predominantly fed milk twice per day to weaning at 5 to 9 weeks old. Rearing systems that reduce the amount of labour reared per calf yet maintain animal welfare and performance standards therefore merit evaluation. The objective of this experiment was to evaluate the effect of rearing dairy-bred beef calves on either a once or twice a day milk feeding system to weaning at 6 weeks old.

Materials and methods Forty four Holstein and Simmental cross Holstein-Friesian beef calves were assigned in a randomised block designed experiment to weaning on either a once a day or twice a day milk feeding system. The calves started the trial at 1 day of age and were individually penned on straw. From days 1 to 4 they were fed colostrum twice per day. The once a day milk fed calves were fed a whey based milk replacer (DM 975 g/kg, 197g crude protein/kg, 283 g/kg Ether Extract [Once a day Watermix, Wynnstay Group plc]) mixed at 200g per litre of water at 40°C once per day. From days 5 to 8 they were fed 2 litres per day, from days 9 to 12 fed 2.5 litres, from days 13 to 28 fed 3 litres and from days 29 to weaning at day 42 fed 3.5 litres per day. The twice a day milk fed calves were fed a whey based milk replacer (DM 936g/kg, 212g crude protein/kg, 179 g/kg Ether Extract [Wynngold Calf Milk Replacer, Wynnstay Group plc]) mixed at 125g per litre of water at 40°C twice per day. From 5 to 7 days they were fed 4 litres per day and from day 8 to weaning milk was fed at 5 litres per day. From day 4 the calves on both treatments received *ad libitum* concentrates (DM 843 g/kg; 207g crude protein/kg DM, 247g NDF/kg DM, 53g ether extract/kg DM, and 290g starch/kg DM [Start 'n' Wean pellets, Wynnstay Group plc]) plus straw and water. The calves were moved into group pens at weaning. The data was analysed by ANOVA with calves blocked according to weight and breed.

Results The calves reared on the twice a day system recorded significantly higher DLWG's to 3 weeks old ($P < 0.01$). There were no other significant differences in calf performance or feed intakes. There were no differences in the health or condition of the calves.

Table 1 Effect of milk feeding system on liveweight (kg) and coat bloom score

	Twice	Once	s.e.d	Sig	
Start weight	46.6	49.7	2.04	NS	
3 week weight	54.0	54.5	2.31	NS	
Weaning weight	69.9	69.4	3.33	NS	
12 week weight	123.1	121.9	5.98	NS	
Coat bloom score at weaning*	2.50	2.34	0.101	NS	

* Coat bloom score scale of 1 = dull, 2 = normal, 3 = shiny

Table 2 Effect of milk feeding system on DLWG (kg)

	Twice	Once	s.e.d	Sig
Birth – 3 weeks	0.354	0.229	0.0459	**
3 weeks - weaning	0.755	0.709	0.0811	NS
Weaning - 12 weeks	1.266	1.250	0.0819	NS
Birth - 12 weeks	0.910	0.860	0.0585	NS

Table 3 Feed intakes (kg/head)

	Twice	Once	s.e.d	Sig
Conc intake (birth – weaning)	27.4	30.6	3.48	NS
Conc intake (birth – 12 weeks)	166.0	170.3		
Milk replacer	22.9	23.0		

Based on the prices prevailing at the time of the study with the twice a day milk replacer costing £1,350/t, once a day milk replacer costing £1,600/t and concentrates costing £172/t, the total feed costs per calf were £59.46 and £66.09, and the feed costs per kg LWG were 77.7p and 91.5p for the twice and once a day milk feeding systems respectively. The time spent attending each calf for feeding, bedding and checking was 116.8 and 58.8 minutes from start to weaning for the twice and once a day systems respectively. With labour valued at £10 per hour it cost £19.46 and £9.80 per calf.

Conclusions Calf growth rates on both systems exceeded the recognised targets for rearing bull calves to 12 weeks old of 115kg (MLC 1999). The calves on the twice a day milk feeding system recorded significantly higher DLWG's from start to 3 weeks. There were no significant differences in live weight at weaning or 12 weeks, or feed intakes between the treatments. Labour inputs were reduced by 49.7% with the 'once a day' system estimated to be worth £9.66 a calf, however feed costs to 12 weeks old were increased by £6.63 reducing the net margin to £3.03 per calf. The benefit of changing to a once a day system however would be negated if a value of £1.20/kg is placed on the extra 4.3kg gained to 12 weeks of age by the twice a day milk fed calves.

Acknowledgement Funding for this study was provided by Wynnstay Group plc.

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The effect of fluctuation in rumen pH on attachment of *Ruminococcus flavefaciens* to dietary substrates as determined by real-time PCR

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Introduction Rumen pH, together with the microbial population, nature of substrates, environmental factors such as temperature and existence of cations and soluble carbohydrates have been suggested as factors governing bacterial attachment to ingested feed particles (Miron *et al.*, 2001). Ruminal pH is one of the most important of these factors, because fibrolytic bacteria are very sensitive to pH change. Fibre digestion decreases at low rumen pH, especially below pH 6.0, as observed previously in studies using continuous cultures of mixed ruminal micro organisms, *in vitro* rumen, and *in situ* techniques. The objective of the present experiment was to investigate the effect of fluctuation in rumen pH on the attachment of *Ruminococcus flavefaciens* to feed particles incubated in the rumen.

Materials and methods Four Holstein steers (300 ± 15kg, body weight) with rumen fistulae, were fed experimental diets (kg of DM/d) differing in their concentrate (155 g CP/kg DM; 30% maize, 34% barley, 8% soybean meal, 5% sugar beet pulp, 10% wheat bran, 12% cottonseed meal, 0.3% CaCO₃, 0.5% mineral and vitamin premix, 0.2% salt) to forage (lucerne hay; 155 g CP/kg DM) ratio (60:40, 70:30, 80:20, and 90:10) in a 4×4 Latin Square design (28 day periods). Steers were housed in individual pens, and fed the experimental diets as a total mixed ration at 0800h and 2000h. Animals had access to drinking water at all times. Ruminal fluid was taken, by suction, via the rumen fistula on days 24 to 28 of each period. The pH of the ruminal fluid samples was measured immediately with a portable pH meter (Metrohm 744, Switzerland) before the morning feed (0.0h) until 8h post feeding at 15min intervals. Feed samples (lucerne hay and barley grain) were dried using a forced-air oven at 96 °C for 48 h, and ground to pass through a 2mm screen. Approximately 1.2g DM of each sample was placed in polyester nylon bags, 3 × 6cm; 48µm pore size, (4 bags per each feed) and incubated in the rumen of each steer for 12h. After removal from the rumen, DNA was extracted from the incubated samples using the QIAamp[®] DNA stool mini kit (Qiagen Ltd, Crawley, West Sussex, UK) following the manufacturer's instructions. *R. flavefaciens* rDNA concentrations were measured by real time PCR relative to total bacteria amplification (ΔΔCt). The 16s rRNA gene-targeted primer sets used in the present study were forward: CGAACGGAGATAATTTGAGTTTACTTAGG and reverse: CGGTCTCTGTATGTTATGAGGTATTACC. Cycling conditions were 95°C for 5min, forty cycles of 95°C for 15s, 61°C for 1min and 72°C for 30s; fluorescence readings were taken after each extension step, and a final melting analysis was obtained by heating at 0.1°C/s increment from 65 to 95°C, with fluorescence collection at 0.1°C at intervals. Data were expressed relative to quantification of the total bacterial population quantified using the primers described by Maeda *et al.* (2003). Data were analysed using the GLM procedure of SAS (y = Mean + Treatment + Animal + Period + Time + Time × Treatment + residual) and the means compared by the Duncan test (P <0.05).

Results Rumen pH at different sampling times is shown in Figure 1. Rumen pH decreased, when the level of concentrate was increased (P <0.05). Concentrations of rDNA in *R. flavefaciens* attached to lucerne hay and barley are shown in Figure 2. Bacterial attachment to both lucerne and barley decreased as the level of concentrate in the diet increased (P <0.05).

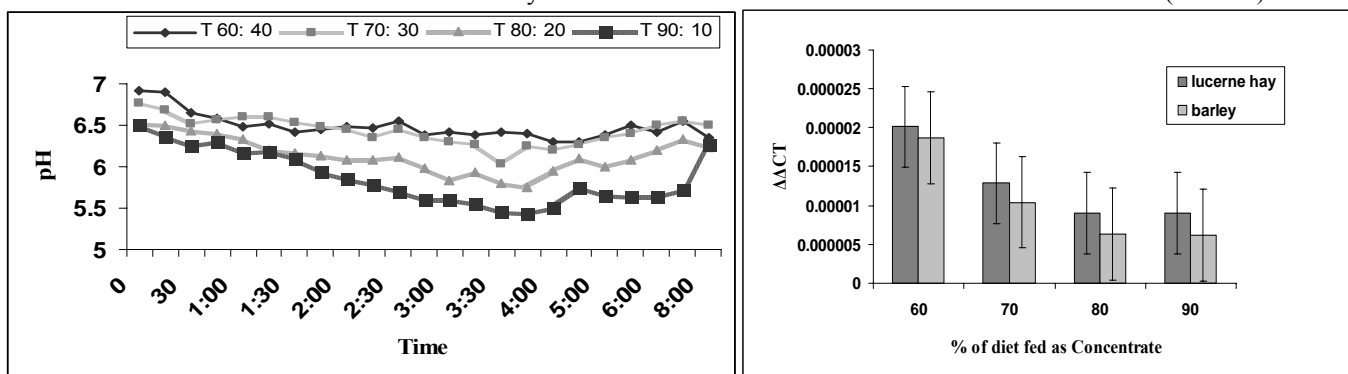


Figure 1 Rumen pH in Holstein steers fed diets differing in concentrate: forage ratios

Figure 2 *R. flavefaciens* (± SD) attached to lucerne hay and barley after 12h incubation in the rumen

Conclusions The results of the present study demonstrated that increasing the inclusion of concentrate in diets caused a decrease in ruminal pH and the population of *R. flavefaciens* attached to substrates. The exact mechanism involved in reduced attachment at low pH is not known. However, Russell and Wilson (1996) suggested that as pH declines, the increase in the transmembrane pH gradient in the bacterium causes a logarithmic accumulation of intracellular fermentation acid anions and hence leads to anion toxicity and product inhibition.

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The effect of fluctuations in rumen pH on *Fibrobacter succinogenes* populations in rumen fluid as determined by real-time PCR

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Introduction The complex symbiotic microbiota in the rumen is responsible for the enzymatic breakdown of plant fibres, an ability the ruminant host animal lacks. This microbiota is highly responsive to changes in diet, age, antibiotic use, and the health of the host animal, and varies according to geographical location, season, and feeding regimen. *Fibrobacter succinogenes* and *Ruminococcus flavefaciens* are considered to be the predominant cellulolytic bacteria present in the rumen (Krause *et al.*, 2003). Ruminal pH fluctuates considerably in a 24h period and is influenced by the intake of fermentable carbohydrate, the inherent capacity of the animal to produce saliva, and rates of utilization and absorption of acids. In beef cattle fed high-concentrate diets, the ability of the animal to buffer the rumen is limited by inadequate salivary secretion. The objective of the present experiment was to investigate the effect of fluctuation in ruminal pH on the population of *F. succinogenes* in rumen fluid.

Materials and methods Four Holstein steers (300 ± 15kg, body weight) with rumen fistulae were fed experimental diets (7kg of DM/d) differing in their concentrate (155g CP/kg DM; 30% maize, 34% barley, 8% soybean meal, 5% sugar beet pulp, 10% wheat bran, 12% cottonseed meal, 0.3% CaCO₃, 0.5% mineral and vitamin premix, 0.2% salt) to forage (lucerne hay; 155g CP/kg DM) ratio (60:40, 70:30, 80:20, and 90:10) in a 4×4 Latin Square design (28-day periods). Steers were housed in individual pens, and fed the experimental diets as a total mixed ration at 0800 and 2000h. Animals had access to drinking water at all times. Ruminal fluid was taken, by suction, via the rumen fistula on days 24 to 28 of each period. The pH of the ruminal fluid samples was measured immediately with a portable pH meter (Metrohm 744, Switzerland) before the morning feed (0.0h) until 8h post feeding at 15min intervals. The samples of rumen fluid taken before the morning feed, and 4h post feeding were stored in liquid N₂ until used for bacterial quantitation by qPCR. DNA was extracted from the samples using the QIAamp® DNA stool mini kit (Qiagen Ltd, Crawley, West Sussex, UK) following the manufacturer's instructions. *F. succinogenes* rDNA concentrations were measured by real time PCR relative to total bacteria amplification (ΔΔCt). The 16s rRNA gene-targeted primer sets used in the present study were forward: GTTCGGAATTACTGGGCGTAAA and reverse: CGCCTGCCCTGAACTATC. Cycling conditions were 95°C for 5min, forty cycles of 95°C for 15s, 61°C for 1min and 72°C for 30s; fluorescence readings were taken after each extension step, and a final melting analysis was obtained by heating at 0.1°C/s increment from 65 to 95°C, with fluorescence collection at 0.1°C at intervals. Data were expressed relative to quantification of the total bacterial population using the primers described by Maeda *et al.* (2003). Data were analysed using the GLM procedure of SAS (y = Mean + Treatment + Animal + Period + Time + Time × Treatment + residual) and the means compared by the Duncan test (P < 0.05).

Results Ruminal pH decreased, from before the morning feed 6.91 (60:40), 6.76 (70:30), 6.52 (80:20) and 6.50 (90:10) to 4h after feeding 6.30 (60:40), 6.20 (70:30), 5.94 (80:20) and 5.50 (90:10), respectively, (P < 0.05). The concentration of rDNA concentration in *F. succinogenes* in rumen fluid is shown in Figure 1. The Population of *F. succinogenes* in the ruminal fluid increased, when level of concentrate was increased (P < 0.05).

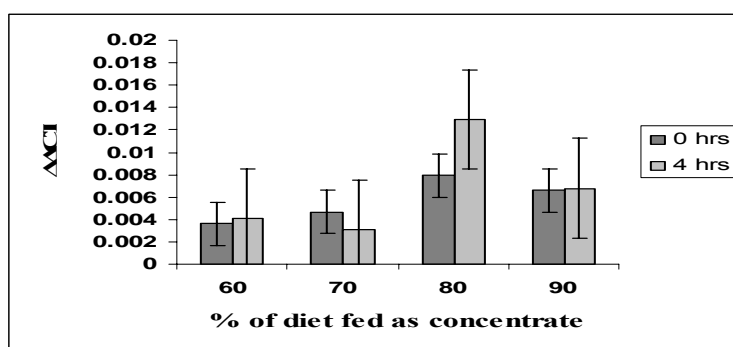


Figure 1 *F. succinogenes* (± SD) in rumen fluid before and 4 h after morning feeding

Conclusions The results of the present study demonstrated that increasing the inclusion of concentrate in diets caused a decrease in rumen pH and an increase in the population of *F. succinogenes* in the free rumen fluid. These results are in apparent contradiction with pure culture studies that suggest that *F. succinogenes* is capable of little or no growth at pH values below 6 (Russell and Wilson, 1996). The reason for this apparent contradiction remains unclear but in the liquid phase *F. succinogenes* numbers are not determined solely by rumen pH.

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Study on the efficacy of *Artemisia* species leaves extract against common nematodes of sheep

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Introduction Parasitic disease remains a major problem to livestock across all production areas of Nepal. 24% of deaths in sheep were reported to be due to internal parasites (Shrestha *et al* 1988) and total economic losses were reported to be about 25% (Lohani and Rasali 1995). The main way of controlling nematode parasite of sheep and goat has been with the use of chemical anthelmintics. However, poor and uneducated farmers are unaware of this method and synthetic anthelmintics are not readily available in the rural village. Moreover, these drugs are very costly. Ethnic people in Nepal are using several plants as medicine. Thus, the aim of the present study was to validate scientifically the use of *Artemisia* species as anthelmintic against the nematode of sheep.

Material and methods Fresh artemisia plants were collected from the surrounding areas of the Institute of Agriculture and Animal Science, (IAAS) Rampur. All the usable plant parts (green leaves and shoots) were weighed and recorded. All the plant parts were cleaned with water and ground with a mortar and pestle, strained through fine muslin cloth and stored in a plastic bag at 4° C until use. A total of 40 sheep of both sexes aged from eight months to two years, weighing 13-24 kg, were used for the experiment. Before the start of the experiment, the animals were confirmed to be naturally infected with mixed species of gastrointestinal nematodes by quantitative faecal examination using a modified McMaster counting technique for counting nematode eggs per gram faecal material (EPG). All the animals were reared under the same environmental and managerial conditions during the experiment. Sheep were drenched with the plant extract, in the morning, at different dose rates. The sheep (n=40) used for the experiment were randomly divided into five groups of eight animals each and assigned to different treatments, which were either untreated, treated with albendazole, or treated with artemisia extract at a dose rate of 6, 8 or 10 g/kg liveweight (lwt). Faecal samples of each group were collected in the morning, starting from day 0 pre-treatment and at day 3, 7, 10, 14, post- treatment and worms eggs were counted by the Modified McMaster Counting Technique. Data were analysed using one way analysis of variance.

Results The results are summarised in Table 1.

Table 1 Efficacy of *Artemisia* leaf extract against common nematodes of sheep

Group no.	Drug used	Mean EPG ± S.E. expt.	Mean EPG ± S.E. after treatment				Efficacy %
			3 rd day	7 th day	10 th day	14 th day	
Group I	Untreated group	1500 ± 300	1600 ± 400	1650 ± 350	1750 ± 350	1800 ± 400 ^a	
Group II	Treated with Albendazole	1400 ± 100	200 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0 ^c	92.85
Group III	Artemisia extract 6g/kg lwt	1260 ± 116.6	800 ± 70.71	720 ± 58.30	640 ± 67.82	580 ± 44.7 ^{abc}	53.96
Group IV	Artemisia extract 8g/kg lwt	1400 ± 70.71	740 ± 50.99	640 ± 50.99	580 ± 37.41	540 ± 24.49 ^{abc}	61.42
Group V	Artemisia extract 10g/kg lwt	1200 ± 70.71	520 ± 37.41	440 ± 24.49	380 ± 37.41	^b 340 ± 24.49 ^b	71.66

mean values with different superscripts were significantly different, p>0.05.

Conclusions It is concluded that *Artemisia spp* show anthelmintic activity against nematodes in sheep. Therefore, its use in ethno-veterinary medicine as an anthelmintic, particularly by farmers who do not have access to modern, commercial anthelmintics is justifiable. However, further research should be conducted to determine the appropriate dose and whether there are any problems with the plant's toxicity.

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Relationship between ewe body condition score and fat and muscle measurements obtained by real time ultrasound

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Introduction In Northeast of Portugal sheep is reared under extensive systems. These systems frequently involve expressive body composition changes due to the storing and mobilization of body reserves, mainly fat. Body condition score (BCS) is the most common way to assess these reserves and the nutritional status of ewes. However due to the subjective nature of BCS, their quality has been questioned and other alternatives has been studied. For cattle there are some studies that use the real time ultrasonography (RTU) to evaluate the BCS (Schwager-Suter *et al.*, 2000; Broring *et al.*, 2003), but this approach was not tested in ewes. Therefore the aim of the present study was to establish a relationship between the BCS and ultrasound subcutaneous fat (SF) and *Longissimus thoracis et lumborum* muscle (LM) measurements.

Materials and methods Five hundred and seventy one ewes of the native Churra da Terra Quente breed (43.6±6.4 kg) were used in this study. All ewes were evaluated for BCS using the approach proposed by Russel *et al.* (1969). Body condition was assessed by 3 assessors according to a scale ranging from 1 to 5 in a quarter unit increments. At same time all animals were scanned with an Aloka real time scanner using a linear probe of 7.5 MHz. Sheep were individually restrained in a crate to minimize movements and ensure that they were standing in a similar stance. The probe was placed perpendicular to the backbone, over the 13th thoracic vertebra and between the 3rd and the 4th lumbar vertebrae. The measurements of subcutaneous fat depth (SFD) at these sites represented two fat depths - SFD13 and SFD34, respectively. The depth of the LM (LMD) was measured over the 13th thoracic vertebra and at the interval between the 3rd and 4th lumbar vertebrae yielding two muscle depths - LMD13 and LMD34, respectively. The images were analysed with Image J software. Data were analysed using SAS (SAS Inst., Inc., Cary, NC). RTU measurements were analysed using a one-way ANOVA with body weight as covariate. Regression between BCS and RTU measurements were established.

Results Least squares means and standard errors of RTU measurements for different BCS are presented in Table 1. The results show that the SF for thoracic and lumbar measurements present similar pattern of variation, presenting an increase around 0.2 mm for each quarter of BSC point for notes between 2.5 and 3.75 followed by an increase around 0.5 mm for each quarter of BSC point for notes superior to 3.75. However, the variation of SF depth measurements is higher than those of LM depth. In fact, for BSC notes between 1.5 and 4.75, SF depths increased 112.4% in SFD13 and 113.6% in SFD34 and LMD increased 24.8 and 32.8%, for the same positions.

Table 1 Least squares means and standard errors of RTU measurements for different BCS.

BCS	n	SFD13 (mm)	SFD34 (mm)	LMD13 (mm)	LMD34 (mm)
BCS1.50	11	3.31±0.41 ^a	3.38±0.31 ^a	18.5±1.01 ^a	17.6±0.92 ^a
BCS1.75	10	3.80±0.22 ^{ab}	3.74±0.17 ^{ab}	19.3±0.57 ^{ab}	18.4±0.45 ^b
BCS2.00	36	3.92±0.14 ^b	3.87±0.08 ^b	19.8±0.29 ^{bc}	19.1±0.26 ^{bc}
BCS2.25	26	4.25±0.17 ^{bc}	4.05±0.10 ^{bc}	19.9±0.36 ^{bc}	19.3±0.28 ^{bcd}
BCS2.50	48	4.50±0.12 ^c	4.29±0.08 ^{cd}	20.1±0.26 ^{bcd}	19.3±0.19 ^{cd}
BCS2.75	27	4.52±0.15 ^{cd}	4.36±0.09 ^{cde}	20.3±0.30 ^{cd}	19.3±0.24 ^{cd}
BCS3.00	71	4.78±0.09 ^{cde}	4.43±0.07 ^{de}	20.3±0.16 ^{cd}	19.9±0.13 ^d
BCS3.25	57	4.87±0.09 ^{de}	4.80±0.08 ^{ef}	20.4±0.21 ^{cd}	19.9±0.18 ^d
BCS3.50	54	5.00±0.10 ^e	4.99±0.09 ^{fg}	20.6±0.17 ^{cde}	20.1±0.16 ^{de}
BCS3.75	58	5.15±0.09 ^e	5.13±0.08 ^g	20.7±0.15 ^{de}	20.5±0.14 ^e
BCS4.00	79	5.53±0.11 ^f	5.44±0.08 ^h	21.7±0.19 ^{ef}	21.1±0.16 ^{ef}
BCS4.25	44	5.88±0.21 ^g	5.76±0.16 ⁱ	22.1±0.40 ^f	21.3±0.37 ^f
BCS4.50	23	6.42±0.32 ^h	6.58±0.24 ^j	22.6±0.62 ^f	22.1±0.58 ^{fg}
BCS4.75	27	7.03±0.84 ^h	7.22±0.75 ^k	23.1±1.52 ^f	23.4±1.55 ^g
Effect		***	***	***	***
R ²		0.752	0.717	0.561	0.540
RSD		1.17	1.25	1.72	1.81

a,b,c,d,e,f,g,h,i,j,k - Means in a column with different superscripts differ ($P < 0.05$), *** $P < 0.001$, R²- coefficient of determination, RSD- residual standard deviation.

Conclusions The BCS is significantly correlated with thoracic and lumbar SF and LM depth measurements using RTU, which indicates that this technique is able to monitor changes on ewe body reserves.

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Effect of sex, degree of maturity and diet of kids from Serrana breed on carcass composition and muscle chemical composition

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Introduction In Portugal consumers prefer low weight carcasses from kids. Some studies on carcass and meat characteristics were conducted with this type of carcasses (Santos *et al.*, 2007). However, there is little information on the effect of increasing the live weight at slaughter by supplementing the diets fed to kids with concentrate in order to maximize meat production. The objective of this study was to evaluate the effect of diet, sex and degree of maturity of kids from Serrana breed on carcass composition and in muscle chemical composition.

Materials and methods Thirty six kids of Serrana breed (18 males and 18 females) were divided in 3 groups according the degree of maturity at slaughter of 20 (M20), 30 (M30) and 40% (M40) of mature body weight. The maturity weight for this breed is 56 kg and 40 kg, for males and females respectively. All animals were fed *ad libitum* on a natural pasture and with hay. Each group was submitted to different nutritional regimes. One with concentrate- 23g/kg live weight (C) and the other without concentrate (W). Animals were slaughtered as they reached the degree of maturity previously established. After slaughter the carcasses were completely dissected in muscle, subcutaneous fat, intermuscular fat and bone according the methodology propose by Fisher and DeBoer (1994). The muscle was analysed and the protein, fat, moisture and ash contents were determined according to the procedures of the AOAC (1990). The statistical analysis was performed by ANOVA module (Statistica V5 program). A fixed model of diet group, sex and maturity degree and appropriate interactions was utilized. No significant diet x sex x degree of maturity interactions were observed for carcass and muscle composition evaluated in this study, therefore only main effects will be presented.

Results In Table 1 shows the mean values for carcass composition and for muscle chemical composition. Diet has no effect on both carcass and muscle composition. Sex and degree of maturity significantly affected the carcass and the muscle composition; males presented higher ($P < 0.05$) muscle content and lower ($P < 0.05$) subcutaneous fat and total fat than females, and animals from group M20 presented more ($P < 0.05$) bone content than animals from group M40. The sex also affected the muscle chemical composition. Males presented higher ($P < 0.05$) moisture and lower ($P < 0.05$) fat and ash contents than females. No significant ($P > 0.05$) differences on protein content were observed between males and females.

Table 1 Mean±standard deviation for proportion of muscle (M), subcutaneous fat (SF), intermuscular fat (IF), total fat (TF), bone (B) in the carcass (g/kg carcass) and moisture, protein, fat and ash of muscle (g/kg muscle).

	Sex		Degree of maturity			Diet	
	Male	Female	M20	M30	M40	C	W
Carcass composition							
M	640.6±28.2 ^a	613.6±28.7 ^b	613.4±30.5	629.7±29.2	638.2±31.0	623.2±24.2	631.0±37.2
SF	39.4±17.2 ^a	65.4±19.9 ^b	59.4±22.1	49.0±24.6	48.9±21.3	55.4±21.4	49.5±24.0
IF	73.8±9.0	80.8±13.1	76.7±14.5	74.1±11.1	81.1±8.2	76.8±9.0	77.8±14.0
TF	113.2±22.9 ^a	146.2±29.2 ^b	136.1±34.4	123.1±33.3	130.0±25.3	132.2±27.1	127.3±34.8
B	220.0±13.2	212.8±13.7	221.3±11.0 ^a	220.2±14.1 ^a	207.7±12.6 ^b	217.5±13.7	215.3±14.1
Muscle chemical composition							
Moisture	728.1±16.8 ^a	711.3±22.8 ^b	721.6±20.3	718.1±22.6	719.5±23.3	722.8±18.3	716.7±24.4
Protein	207.5±10.5	215.5±21.0	209.6±19.6	208.4±17.8	216.5±12.7	210.2±17.9	212.9±16.2
Fat	21.7±6.5 ^a	28.1±8.8 ^b	22.2±9.7	23.6±7.9	28.9±6.0	25.4±7.6	24.4±9.1
Ash	16.2±3.0 ^a	17.9±1.6 ^b	18.6±0.8 ^a	16.7±2.5 ^{ab}	15.9±3.0 ^b	17.3±2.4	16.9±2.6

Means in the same row with different letter are significant difference ($P < 0.05$).

Conclusions The degree of maturity and sex significantly affected carcass composition of kids from Serrana breed. However, sex was the only factor that affected the muscle chemical composition. It was also concluded that, when the degree of maturity varied from 20 to 40%, the supplementation of diets fed to kids from Serrana breed with concentrate has no effect on both carcass and muscle composition.

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Breeding for resistance to footrot – using hoof scoring to quantify footrot in sheep

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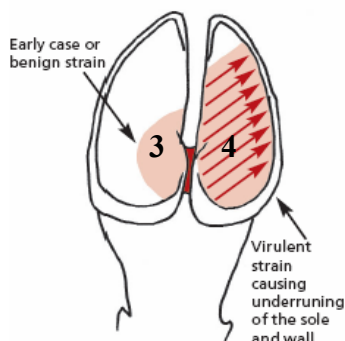
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Background Footrot is the main cause of lameness that adversely affects the welfare and productivity of sheep so measures to control it will bring both economic and welfare benefits. The use of breeding strategies to manage footrot is a cumulative and sustainable solution that may contribute to its control. As part of a research programme to investigate genetic resistance to footrot, the aim of this paper is to assess the usefulness of foot score categories to quantify footrot in sheep, to identify key environmental factors that affect footrot and to assess the repeatability of different foot scorers on separate scoring occasions.

Methods A foot scoring method was developed and tested based on Egerton and Roberts (1971). Five foot lesion categories were used. These were: 0 = no footrot (normal foot); 1 = mild inter-digital dermatitis ('scald') with some loss of hair, slight to moderate inflammation confined to inter-digital skin and may involve erosion of epithelium; 2 = more extensive inter-digital dermatitis and necrotising inflammation of inter-digital skin. 3 = Severe inter-digital dermatitis and under-running of the horn of the heel and sole to 1cm; 4 = Severe inter-digital dermatitis and under-running of the horn of the heel and extending >1cm towards the walls of the hoof (Figure 1). A single commercial flock of 100 mixed-age (2 to 6) mule ewes that grazed in two ryegrass paddocks was used for this trial and two trained foot scorers independently assessed each hoof i.e. left front and hind, and right front and hind (LF, LH, RF, RH respectively). This was repeated on the same day so that each ewe had 16 separate foot scores resulting in a data set of 1600 separate hoof assessments. The data were analysed for each hoof separately, for the sum of footrot scores over the 4 hooves (SUM_FR) and also as a binomial trait with a ewe awarded '1' if any hoof scored >0, and '0' if it scored a zero on all 4 hooves (ZERO-1). The continuous data were analysed using the GLMM and REML directives in Genstat (2007) assuming normal distribution (log-transformed data yielded similar results so un-transformed data were used here). ZERO-1 was analysed using a binomial distribution and a logit link function. Residual correlations (after fitting ewe age; 2 vs 3-6 years, grazing paddock (n=2), scorer and scoring occasion) for SUM_FR were used to assess agreement between scorers and scoring occasions.

Results The average prevalence of footrot (i.e. any score >0) was 15%, and of scores >1 was 12%. The % distribution and score means for each hoof is shown in Table 1. Table 2 shows mean foot scores (s.e.d.) according to hoof and ewe age.

Figure 1 Foot scores 3 and 4



Courtesy of Agnes Winter

Table 1 Percent distribution and mean foot scores according to hoof and score category

	0	1	2	3	4	Mean
LF	22.3	0.7	0.8	0.4	0.9	0.29 ^a
LH	20.8	0.8	1.3	0.7	1.4	0.45 ^b
RF	22.6	0.6	0.9	0.4	0.4	0.22 ^a
RH	19.4	0.8	0.8	0.9	3.1	0.71 ^c

Table 2 Mean foot scores according to ewe age

	2-yrs	3-6 yrs	s.e.d.
LF	0.15 ^a	0.52 ^b	0.089
LH	0.08 ^a	0.69 ^b	0.107
RF	0.14 ^a	0.43 ^b	0.072
RH	0.18 ^a	1.3 ^b	0.132
SUM_FR	0.37 ^a	2.9 ^b	0.287

Means not sharing a common superscript are significantly different ($P < 0.05$)

Both scorers agreed fully (for SUM_FR) 71% of the time and for SUM_FR +/- 2 this rose to 91%. The residual correlation between scorers and scoring occasions for SUM_FR was the same at 0.87. Restricting the data set to non-zero values made little difference to these correlations. Differences were detected for ewe age, with 2-year-old ewes having significantly lower footrot scores than 3-6-year-olds (Table 2). Grazing paddock was a significant factor with mean foot scores of 0.47 vs 0.36 (s.e.d. 0.056).

Significant differences were seen between hooves, with hind legs being significantly more likely to be infected than front legs (Table 1). No significant differences were detected between scorers or between scoring occasions for any of the traits and different analytical methods used ($P > 0.05$).

Conclusions The scoring system described here for footrot is a repeatable and reliable method to differentiate clinical footrot lesions and the agreement of trained foot score assessors is high. Ewe age and grazing location were identified as being important environmental factors that should be accounted for in genetic analyses. This method can be used in structured genetic studies to estimate genetic parameters (Nieuwhof *et al.*, 2008), breeding values and to validate genetic markers for footrot resistance. It can also be used by farmers as a management tool to control footrot.

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Comparison of fixed and random regression test day animal models in genetic evaluation of Iranian Holstein heifers for somatic cell score

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Introduction Mastitis has been one of the major diseases in dairy herds inducing economic costs associated with decreasing milk yield and its quality as well as increasing health care practices. In response to bacterial infection, the concentration of somatic cells increases in the milk of dairy cows (Boettcher *et al.*, 2007). Somatic cell count has been widely utilised as an indicator trait for mastitis. Somatic cell count, like milk yield, varies with stage of lactation and more accurate genetic evaluation of dairy cows could be obtained when a test day model is used (Haile-Mariam *et al.*, 2001). The main aim of this research was to compare fixed and random regression test day models in genetic evaluation of Iranian Holstein first lactation cows based on their predicted breeding value for somatic cell score.

Material and methods The data consisted of 101,147 monthly test day somatic cell counts collected from 13977 Iranian Holstein heifers calving between 2002 and 2006 and distributed in 184 herds. The number of sires, dams and total animals in the pedigree file were 871, 12882 and 27171 respectively. Natural logarithm was applied to transform somatic cell counts (SCC) to somatic cell scores (SCS). Fixed and random regression test day models were used to predict breeding value of individual cows. In both models, contemporary group of province – herd - year of calving - season of production, age of cows (linear and quadratic covariables) and percentage Holstein genes (linear and quadratic covariates) were included as fixed environmental factors. To take account of phenotypic, genetic and permanent environmental variation of somatic cell scores over the course of the lactation, Legendre orthogonal polynomials of level ($k=0$) and cubic order ($k=4$) were utilised in the fixed and random regression test day models respectively. The average SCS, first calving age and Holstein gene in the data set were 4.47, 25.7 (months) and 94.4% respectively. The models were fitted to the data using WOMBAT (Meyer, 2006) and DXMRR (Meyer, 1998) programmes.

Results Average predicted breeding value of cows with records for individual lactation months resulted from random regression test day model along with the predicted breeding value obtained from fixed regression test day model are presented in Table 1. Generally, the average predicted breeding value showed an increase from the beginning towards the middle of the lactation and after decreasing at months 6 and 7, it increased towards the end of the lactation. Paired sample t-test showed that the average predicted breeding value of random regression test day model was statistically significantly ($p<0.001$) greater than the average predicted breeding value of fixed regression test day model. The results obtained in the present research revealed that Spearman rank correlation between predicted breeding value of two models was 0.97 which was statistically significant ($p<0.001$). The magnitude of phenotypic, additive genetic, permanent environment and error variances from fixed regression model were 1.4826, 0.0436, 0.3625 and 1.0765 respectively.

Table 1 Average predicted breeding value for somatic cell score based upon random regression and fixed test day models

Month of lactation											
1	2	3	4	5	6	7	8	9	10	ARPBV ¹	AFPBV ²
-0.0063	-0.0014	0.0015	0.0029	0.0034	0.0033	0.0031	0.0032	0.0041	0.0061	0.002	0.0008

¹ARPBV = Average predicted breeding value based on random regression test day model

²AFPBV = Average predicted breeding value based on fixed regression test day model

Conclusion The results obtained in the present study revealed that predicted breeding value of somatic cell score in Iranian Holstein heifers was not constant rather it varied over the period of lactation. In contrast to random regression test day model, constant variances were assumed for genetic, permanent and temporary environmental effects in fixed regression test day model which resulted in a constant predicted breeding value of somatic cell score over the lactation trajectory. Although there was a high rank correlation between two models, the average predicted breeding value for SCS resulted from random regression test day model was significantly greater than that of fixed regression test day model suggesting the former should be used as genetic evaluation of Iranian Holsteins for somatic cell score are to be practiced.

Acknowledgements The Centre of Animal Breeding of Iran is acknowledged for supplying the data used in this study.

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Investigation on effects of selection system in parental generation on efficiency of reproduction traits in three Chinese lines of silkworm

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Introduction Silkworm is an important industrial insect with many unique characters. Many reports showed that many traits in silkworm have heterotic effects (Rajanna and Puttaraju, 1998). Newly evolved silkworm lines and hybrids need to estimate these effects (Kumar *et al.* 1994). The use of selection for genetic and economic improvement of traits in the shortest time will have the highest improvement. To date, selection of parental generations for silkworm egg production in next generation, based on phenotypic characterizations without individual records. Although reproduction and production traits record individually has expensive costs, however it expected selection based on individual records can improve offspring performance, especially if these economical traits have relatively high heritability. There are three Chinese pure and commercial lines at Iran. Since these lines have different phenotypic and genotypic characterizations, thus their response to individual selection system can be different. Therefore, in this experiment the effect of selection on reproductive characters on Chinese lines during three years was investigated.

Materials and methods For study of effects of selection on reproduction traits in three Chinese lines silkworm including 32, 104 and 110, these stocks were reared in 8 replications, each ones included 250 larvae and maintained at 3 alternative generations using standard methods and recommended procedures. All families with superior parents for cocoon weight (24 batches) along with all families from ordinary parents (24 batches) were employed. These pure lines were reared under standard conditions until end larval duration. The data of reproduction performances in their offspring at three years and two seasons were recorded, pooled and analysed. All studied characters, were recorded and analysed. Collected data were subjected to statistical analysis of variance test using $Y_{ij} = \mu + L_i + e_{ij}$ model (Y_{ij} : record; μ : trait average; L_i : line effect e_{ij} : residual effects) to find out the low significant difference between these three lines.

Results In Table 1 and Figure 1, the experiment results are summarized. The difference of studied traits between two groups (individual selection base on weight cocoon and randomized selection) were showed. From obtained results, it was showed that individual selection can affected on reproduction traits in future generation. The highest and best un-hatched eggs, un-fertilized eggs and un-fertilized eggs percentage belonged to pure line 32. Thus breeding programs and individual selection for cocoon weight at Chinese lines can improve total reproduction traits in some lines. In previous studies, it was reported that pure examined races had high combining ability. The reproduction traits are important for egg production factories of silkworm and due to their high heredity; the efficiency of direct selection of them is very high. To look at the combining ability and a high heterosis of breed races make usage of these races possible in race correctional programs. Furthermore, the identifying of best selection index using economical traits is very important.

Table 1 The effect of selection system (selected group-randomised group) for reproductive traits among three different lines[†]

Line	Hatched Larvae	Unatched eggs	Unfertilized eggs	Hatched eggs percentage	Un-hatched eggs percentage	Unfertilized eggs percentage
32 Line	68.040±4.23 ^a	-7.000±0.14 ^a	-9.333±0.8 ^a	1.043±0.17 ^a	0.760±0.008 ^a	-1.804±0.07 ^a
104 Line	3.210±0.98 ^b	-4.333±0.06 ^b	-1.333±0.12 ^b	0.712±0.05 ^a	-0.938±0.009 ^a	0.225±0.08 ^a
110 line	74.780±6.47 ^a	-6.130±0.07 ^b	-4.913±0.11 ^a	2.631±0.13 ^a	-1.306±0.16 ^a	-1.326±0.05 ^a

[†] In each column, mean (± SEM) with different letters are significantly different ($P < 0.05$).

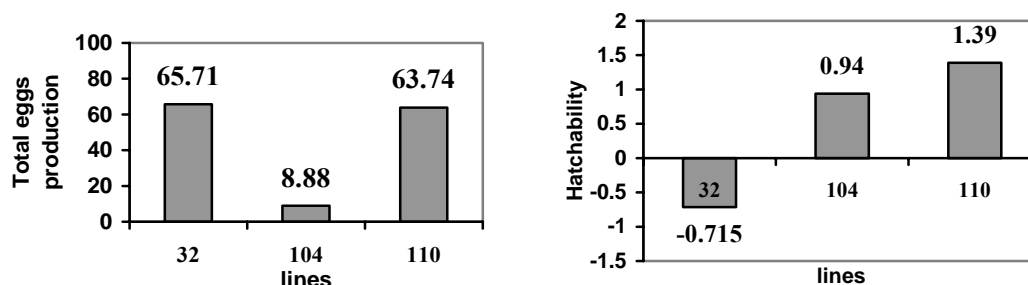


Figure 1 The effect of selection system on total egg and hatchability percentage in three different lines

Conclusions Individual selection system can be applied in line 32 for production system satisfactorily. Furthermore, selection on the basis of a combination of survival, reproduction and yield are suggested as the best system for selection. This tool is very important for the increasing of hybrids efficiency in silkworm lines.

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The frequency of calpain regulatory gene polymorphism in Iranian native Karakul sheep

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Introduction Selection on base marker –assisted selection is one of new methods that may improve accuracy and progress of selection in animal breeding. Genetic variants of ovine calpain gene relationship on growth and meat tenderness examined in many researches. This is a candidate gene in meat tenderization. It is located on ovine chromosome 14 and plays important roles in formation of muscles, degradation and meat tenderness after slaughter. It may be beneficial to use calpain genotypes classified in marker assisted selection programs to improve growth traits of lambs and meat tenderness. (2, 1)

Materials and methods In this study approximately 5 mls of venous blood were collected from eighty eight pure breed karakul sheep and genomic DNA were isolated from leukocytes (DNA Isolation kit Diatom). The ovine m-calpain regulatory gene, exon 5 and 6 which included intron (CAPN456), was amplified with primers which were designed according to the published bovine nucleotide cDNA sequence (Gene bank accession No. J05065). The polymerase chain reactions (PCR) were performed using a commercial kit of Gene Pak™ PCR Core. PCR condition was 200μM MgCl₂, 200μM dNTP, 10-20 pM of each primer, 50- 100 ng of genomic DNA and 1 U Taq DNA polymerase, and H₂O up to a total volume of 25 μl. The reaction was performed using Thermal cycler Biometra, (2000). Preliminary denaturizing was performed at 95°C for 5 min, followed by 35 cycles; denaturizing at 94°C for 45 sec., annealing at 59°C for 1 min., polymerization at 72°C/1.5 min., and a final extension step of 72 °C for 10 min. For the genotyping of all loci, the sample was diluted with 12 μL of running buffer. Running buffer was prepared by mixing: 800μl formamid, 100μl bromophenol blue 1%, 100μl xylenecyanol 1%, 2μl 0.5 M EDTA and 1μl 10M NaOH. After heating at 95°C for five minutes, amplification products were immediately placed on ice. Polymorphisms were detected by SSCP with 8% acrylamid non denaturing gels with 10 % glycerol. The mixture was electrophors for 3-4 hours at 250 V and 10°C. DNA fragments were visualized using silver staining.

Results Under the SSCP analysis condition, different conformations are then separated by gel electrophoresis on non-denaturing condition. A calculated allelic frequency A and B was 0.85 and 0.15 respectively .A allele frequency was observed at CAPN456 locus. Co-dominant inheritance of the genotype and allele were observed in Iranian native of Karakul sheep .The genotype frequency in karakul sheep was 70/3%, 29.6% and 0.0% for AA, AB, and BB respectively. BB genotype was not detected in this station (see Figure -1). In spite of X² test, G test confirmed the Hardy-Winberg equilibrium in this population. Observed heterozygosity (Ho), expected heterozygosity (He), Nei expected heterozygosity, and average heterozygosity of CAPN locus for karakul sheep were low.

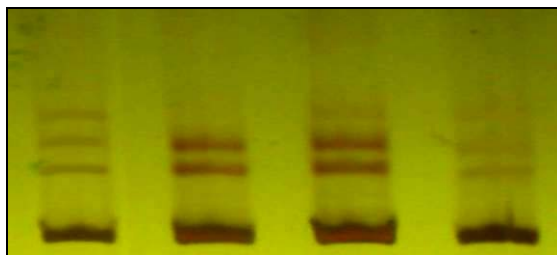


Figure 1 _SSCP Analysis for the 190 bp fregment of Axon 5 and 6 of Calpain Regulatory gene

Conclusion PCR-SSCP analysis of CAPN gene is useful for detection of SNPs. Many studies reported that deference in the potential proteolyses activities of a calpain system result in difference in the rate and extent of postmortem tenderization. Thus, it is possible to use varation at the CAPN locus could be use to predict growth rate and meat tenderization in marker- assisted selection program.

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Relationship between leptin gene polymorphism with economical traits in Iranian Sistani cattle

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Introduction Leptin is a 16 Kilo Dalton protein that is synthesised by adipose tissue and it is involved in regulation of feed intake energy balance, fertility and immune function. In cattle, the leptin gene is located on chromosome 4. It consists of 3 exons and 2 introns that only 2 exons translate into protein. Leptin treatment of animals has been shown to cause a decrease in food intake, body weight loss, fat depot weight loss and increase in energy metabolism therefore leptin not only causes reduced food intake but the potential body weight losses are enhanced due to an increased metabolic rate. Leptin may be involved in regulating reproduction in that it may also act as the signal to the reproductive system that sufficient body fat exists to support a successful conception and pregnancy (Fruhbeck *et al.*, 1998). Lagonigro *et al.* (2003) reported association of five SNP bovine leptin genes with feed intake and fat-related traits. Liefers *et al.* (2002) reported that heifers with the AB genotype produced 1.32 kg/d more milk and consume 0.73 kg which is 2 times more food compared with the AA genotype. They suggested that B allele could yield a higher milk production without negatively affecting energy balance and fertility. The aim of this study was to estimate the frequency of the leptin gene and its relationship between its polymorphisms with variations in growth and age at successful insemination in an Iranian native cattle, Sistani.

Material and methods In this experiment, blood samples were collected from 103 Iranian Sistani cattle randomly. They were obtained from Animal Breeding Station in Zahak district of Zabol city. Blood was collected on K₂EDTA tubes and stored either at -20 °C for few weeks or of -70 °C for several months. DNA extraction was done on the blood samples using guanidium thiocyanate-silica gel. DNA concentration was calculated by spectrophotometry by taking the optical density at 260 nm. A strategy employing polymerase chain reaction (PCR) was used to amplify a 422 bp fragment from blood DNA. PCR condition were 2.25mm MgCl₂, 200µm dNTP, 1µm of each primer, 50- 100 ng of genomic DNA and 0.2 Taq DNA polymerase the first cycles of the was 3 minutes at 94°C followed by 35 cycles of 45 seconds at 94°C, 45 seconds at 60°C, 45 seconds at 72°C and ending with a 10 minute extension phase at 72°C. PCR product for each sample was digested with 5 U of *Bst*MB1 for 6 hours at 65°C. Data on growth and reproduction traits collected from Sistani cattle and analysis with JAMP 4/0/4 . Traits included growth and age at successful insemination.

Results Digestion of amplicons with *Bst*MB1 revealed two alleles: Allele A had 2 fragments 390 and 32bp and allele B had 3 fragments 302, 88 and 32. Frequencies of genotypes were 0.77, 0.22, 0.01 for AA, AB and BB respectively. Allelic frequencies were 0.88 and 0.12 for A and B, respectively. The populations were in Hardy-Weinberg equilibrium. The growth and age at successful insemination traits were affected by the genotypes. Cattle with AB genotype had higher weight at 9 and 12 month of age than the AA genotype (P<0.01). Also the AB genotype demonstrated the highest values for age at conception (P<0.05).

Table 1 Least sq mean, std error effect of animal genotype on growth and age at conception traits in Sistani cows

Trait	AA		AB	
	LSM	SE	LSM	SE
Weight at 9 month (kg)	117.80 ^a	± 0.99	133.85 ^b	± 1.63
Weight at 12 month (kg)	140.28 ^a	± 1.19	155.80 ^b	± 1.74
Age at conception (day)	1117.98 ^a	± 9.44	1154.78 ^b	± 15.07

^{a, b} Means in each column with different superscripts are significantly different (P<0.05)

LSM = Least squares mean SE = Standard error

Conclusion The AB genotype had high positive significant effect on weight at 9 and 12 month of age than the AA genotype (P<0.01). Also the AB genotype demonstrated the highest values for age at conception (P<0.05).

Acknowledgments

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Comparison of linear and non-linear methods in prediction of second parity milk yield in dairy cattle based on first parity production data

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Introduction Milk production in dairy cattle controls by various linear and non-linear factors, and dairy production is influenced by interaction between genetic and environmental agents (Kominakis *et al.*, 2002). Many current models in prediction dairy production assume a linear relationship between dependent and independent data. Artificial Neural Network (ANN) can be used to implement a linear or non-linear relationship for prediction. ANN is made up of set of neurons, to consider the process input data and the corresponding output; to elicit non-linear relationship between input and output data. The parallel processing in ANN is specifying close acquaintance for this system (Lacroix *et al.*, 1995). The objective of this study was prediction of second parity milk yield (MY) based on first parity records by ANN, and comparing with current linear model like multiple linear regression (MLR).

Material and methods From a total of 1,880 records, 1,850 records were used for training and verification of ANN and MLR models, and 30 randomly selected records of the rest were used for simulation. The input variable form first parity were included: %fat 305-d, milk yield 305-d (kg), cumulative fat yield (kg), cumulative milk yield (kg), milking times (2X, 3X), average daily milk yield (kg), parity, calving season (3-mo), number of records, age at calving, and output variable included milk yield 305-d (kg) from second parity. Matlab and SAS software were employed to run ANN and MLR analysis, respectively. The criteria used to compare the result of ANN and MLR prediction with the actual observed data were, (1) Pearson's correlation (r_p), (2) adjust coefficient of determination (R^2_A), and (3) root mean square error (RMSE). The t-test was used for comparison between observed and predicted means.

Results The t-value for comparison between observed and predicted values for ANN was lower than MLR. There was no significant difference between both ANN and MLR predictions and the observed values ($p>0.05$). However ANN mean data had less deviation to observed data rather than MLR mean to observed data. The high correlations showed that the predicted average for milk yield is close to the observed values. Quality parameter RMSE was lower and R^2_A was higher for ANN relative to MLR. %RMSE for ANN was lower than MLR model. %RMSE was used as an indication, percent of deviation of prediction value relative to observed value. Predicted value of ANN shows a lower deviation from the observed data (Table1). However, there was a slight gap between ANN and MLR prediction values.

Table1 t-value and qualities parameter for ANN and MLR methods.

	t-value	r_p	R^2_A	RMSE	%RMSE
ANN	-0.20 ^{ns}	0.825	0.637	817.48	10.9
MLR	-0.46 ^{ns}	0.737	0.327	933.93	12.5

ns: Not significant at ($p>0.05$).

Conclusion The presented results illustrate that ANN model has slightly more ability in the prediction of MY relative to the MLR model. Since ANN can be used for implementation of non-linear relationships between input and input-output variable during prediction procedures, this may led to easier and slightly more accurate ANN prediction. Lower R^2_A for MLR reveals the used input variables may not explain the change in MY properly. Moreover, it is shown that only the chosen independent variables could not explain the changes in the dependent variable by MLR method. r_p supports the fact of ANN fitness for predicting the biological process. Thus in MLR model adding new data require a new statistical model and estimate new parameters, while ANN can be adapted with any new data added to the network (Gerzesiak *et al.*, 2006) which make using ANN simpler than MLR. These findings demonstrate the relative advantage of ANN in comparison with MLR.

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The effect of *Pit-1* gene polymorphism on some of the milk yield traits of Iranian Holstein dairy cows

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Introduction In last decade molecular marker information has been directly used in animal breeding programmes. It is expected that molecular markers could speed up the rate of genetic improvement by up to 30% (Kashi *et al.* 1990). Genetic markers which are related to genes and traits of interest could be used as a marker for selection. It is obvious that milk and reproduction traits are controlled by many genes. However, finding a candidate gene as a genetic marker which may have good influence for increase milk yield is quite important in dairy breeding industry. Some of genetic polymorphism in candidate genes which have significant effects on production traits is widely used. In this study *Pit-1* transcription factor gene was used as a candidate gene. Transcription factor *Pit-1* gene positively regulates the expression of growth hormone, prolactin and thyrotrophin-beta subunit genes (Zhao *et al.* 2004). It also plays a role in pituitary cell differentiation and proliferation. The aims of this study were to find the role of *Pit-1* polymorphism on milk yield, fat and protein percentage and to study the usage of *Pit-1* gene as a candidate gene for milk production traits in dairy cattle.

Materials and methods Random blood samples of 262 cows from 4 different dairy farms in Isfahan province were collected for extracting DNA. Pedigree and yield information of sampled cows were collected from dairy farm database. Using a PCR reaction with these primers (5'-AAACCATCATCTCCCTTCTT-3') and (5'-AATGTACAATGTGCCTTCTGAG-3') as described by Woollard *et al.* (1994) a part of *Pit-1* gene (451 bp) was amplified. Amplified fragments were affected by *HinfI* enzyme for digestion. Non digested fragment (451 bp) indicates the AA genotype while none digested (451 bp) and two digested fragments (244 bp and 207 bp) indicated the AB genotype. However, production of only two digested fragments (244 bp and 207 bp) indicates the BB genotype. Then, the frequencies for each genotype as well as allelic were calculated using SAS software. Covariance analysis was performed by GLM proc of SAS considering genotype and herd as fixed effects and calving interval as a co-variable in the model.

Results The frequency of A and B alleles were 0.27 and 0.73, respectively. The genotypic frequencies of AA, AB and BB were calculated to be 0.04, 0.45 and 0.51, respectively. The genotypic frequencies of the collected samples for *Pit-1* gene were in disagreement with Hardy-Weinberg equilibrium ($p < 0.01$). The genotype was a significant source of variation for fat and protein percent ($p < 0.1$) while total milk yield (kg) and standard milk yield were not affected by the genotype. Fat and protein percentage were highly influenced by Herd management ($p < 0.01$), but total milk yield (kg) and standard milk yield (305d, 2x) have not been affected by this factor. The only considered co-variable in the model (calving interval) has been effective on considered traits except on the protein percentage. Standard milk yield of AB genotype cows was 204 and 81 kg higher than AA and BB homozygote ones, respectively. The percentages of fat and protein were significantly higher in AB cows compare to BB genotypic group.

Table 1 Effect of genotype, herd and calving interval on milk traits

Variation Source		Total milk (Kg)	Standard milk (305d, 2x)	Fat (%)	Protein (%)
	mean	10623±140.26	8618±72.95	2.80±0.03	2.60±0.03
Genotype (mean±SE)	AA	9937±589 ^a	8433±383 ^a	2.70±0.17 ^{ab}	2.53±0.12 ^a
	AB	10659±171 ^a	8637±111 ^a	2.83±0.05 ^a	2.57±0.03 ^a
	BB	10583±162 ^a	8556±105 ^a	2.68±0.05 ^b	2.46±0.03 ^b
Herd (mean±SE)	1 st	10219±252 ^a	8667±164 ^a	3.03±0.07 ^a	2.90±0.05 ^a
	2 nd	10227±333 ^a	8641±216 ^a	2.67±0.10 ^b	2.21±0.07 ^c
	3 rd	10425±288 ^a	8338±187 ^a	2.70±0.08 ^b	2.53±0.06 ^b
	4 th	10701±309 ^a	8523±200 ^a	2.56±0.09 ^b	2.45±0.06 ^b
Calving interval	b	19.775*	3.063*	0.00007*	-0.00008

*= $p < 0.05$. ^{a-c} means within sub-column with different letters are significantly different $p < 0.05$.

Conclusions Overall, it could be concluded that the frequency of B allele was higher than A among sampled animals. There were some herd effects which shows management effects for the percentage of fat and protein but there was no significant effects for milk yield. Total Kg milk yield of AB genotype bearing animals was slightly higher than the other genotypic groups while BB genotype animals were performed better for fat and protein percentages.

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Evaluation of genetic and phenotypic parameters for weight at different ages in Karakul sheep

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Introduction Estimation of variance components is an important step in the development of animal breeding programs. The genetic and phenotype components of weight traits have been reported for different breeds. The heritability of birth weight in Targhee sheep is 0.22 (Bromley *et al.*, 2000) and heritability of weaning weight in Suffolk sheep is 0.19 (Notter *et al.*, 1998). Research shows that the additive genetic effect in early life is low (Nasholm *et al.*, 1996). The objective of this study was to predict additive genetic effect, maternal genetic effect and heritability of lamb body weight in Karakul sheep.

Material and methods Body weight at birth weight (BW), three month weight (3W), six month weight (6W), nine month weight (9W), yearling weight (YW), average daily gain from birth weight to three month weight (ADG BW-3W) and average daily gain from three month to yearling weight (ADG 3W-YW) were considered as traits for prediction of genetic parameters. 3430 records of Karakul sheep were used. Fixed effects that influence these traits (age of dam, sex of animal, birth type, age of ewe, birth year and birth month) processed in Microsoft Excel and analyzed with JMP 4.0 under multiple regression method. Characteristics of records are given in Table 1. Genetic parameters were estimated by using REML method under animal model. Different models of DFREML were applied for this purpose. The best model for each trait was selected with likelihood ratio test. The best used models were model I; $Y=Xb+Za+e$ and model II; $Y=Xb+Za+Wp+e$. In these models Y is vector of observation; b is vector of fixed effect; a is vector of additive genetic effect; p is vector of maternal environment genetic effect; e is vector of residual effect; X, Z and W are matrices due to fixed and random effects.

Table 1 Statistical Characteristics of records

Character	BW	3W	6W	9W	YW	ADG BW-3W	ADG 3W-YW
Mean (kg)	5.15	24.15	32.50	39.44	43.62	0.211	0.078
Standard deviation (kg)	0.79	5.04	5.40	6.72	7.58	0.048	0.023
Coefficient of variation (%)	15.33	2.087	16.61	17.04	17.37	22.74	29.48
Number of records	3430	2986	2837	2346	2474	2870	2440
Number of fixed effects	4	4	5	4	5	4	4
Best model	II	II	I	I	I	II	I

Results Variance components and genetic parameters are presented in Table 2. This study shows that additive genetic effect and maternal environment genetic effect have influence on lamb growth especially in early life. Moreover the proportion of additive genetic effect at the first stage of life is low and maternal environment genetic effect is important especially in ADG BW-3W.

Table 2 Genetic Parameters and Variance component estimation of different weights

Trait	σ^2_p	σ^2_a	σ^2_c	σ^2_e	h^2d (SE)	c^2 (SE)
BW	0.43	0.09	0.07	0.24	0.23 (0.08)	0.176 (0.076)
3W	15.31	3.18	2.10	10.03	0.2 (0.03)	0.136 (0.053)
6W	14.99	3.59	-	11.40	0.23 (0.07)	-
9W	21.73	6.45	-	15.28	0.29 (0.03)	-
YW	23.84	7.32	-	16.52	0.3 (0.055)	-
ADG BW-3W	1747	378.7	259.3	1108.8	0.21 (0.044)	0.14 (0.07)
ADG 3W-YW	289.1	34.84	-	254.21	0.12 (0.032)	-

σ^2_p =phenotypic variance; σ^2_a =additive genetic variance; σ^2_c =maternal environment genetic variance

σ^2_e = residual variance; h^2d = heritability; c^2 = ratio of maternal environment variance to phenotypic variance

Conclusion additive genetic effect and maternal environment genetic effect have influence on BW, 3W and ADG BW-3W moderately. But after weaning weight (3W), additive genetic effect is more significant than maternal environment genetic effect. The results are in agreement with other reports that showed with increasing age of lambs after weaning weight, additive genetic effect and heritability increase and maternal environment genetic effect decrease.

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Effect of cross-breeding of dairy cows on energetic efficiency and energy partitioning

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Introduction Breeding programmes for the Holstein-Friesian have historically focused on improved milk production with little emphasis on functional traits such as fertility or disease resistance (Yan *et al.*, 2006). Recently, a major breeding programme has been adopted in Northern Ireland using the cross-breeding technique (Holstein cows x Jersey sires) with the aim of improving fertility and disease resistance of dairy cows whilst maintaining milk production capacity. The objectives of the present study were to evaluate possible breed differences in the efficiency of energy utilisation between Holstein and Jersey-Holstein dairy cows offered mixed diets of grass silage with a low or high level of concentrates.

Materials and methods Eight Holstein and 8 Jersey-Holstein first lactation cows were used in a 4 period (6 weeks/period) changeover study. Each breed was offered *ad libitum* mixed diets of grass silage with 2 levels of concentrates (0.30 vs. 0.70 kg/kg DM), so the dietary concentrate levels for each breed were either 0.30, 0.70, 0.30 and 0.70 kg/kg DM or 0.70, 0.30, 0.70 and 0.30 kg/kg DM during the 4 periods. The animals were on experiment from approximately 28 days of lactation and there was a 10 week break interval between periods 2 and 3. During the pre-experimental period and the break interval, all animals were offered the same silage and concentrates with a ratio of 0.50/0.50 (DM basis). The animals were housed in cubicle accommodation and then transferred to metabolism units on the 11th day from the end of each period. Faeces and urine outputs were collected from day 3 to 8 in metabolism units. From day 9 to 11, cows were placed in indirect respiration calorimeter chambers with gaseous exchange measured during the final 48 hours. Data were analysed by ANOVA to examine effects of breed and dietary concentrate level, using experimental period as block.

Results There was no significant treatment interaction on animal performance or energy metabolism. On average across the 2 concentrate levels, cow breed had no significant effect on live weight or milk yield, but Jersey-Holstein cows produced a significant higher DM intake ($P < 0.01$), body condition score ($P < 0.05$) and milk concentration of fat and protein ($P < 0.001$). Energy corrected milk yield (milk energy output/energy content per kg standard milk) was significantly higher with Jersey-Holstein than Holstein cows ($P < 0.05$). As expected, the high level of concentrates resulted in an increased GE intake, energy outputs in faeces, urine and milk and heat production ($P < 0.01$ or 0.001 , Table 1). In comparison with Holstein cows, Jersey-Holstein animals had a significantly higher GE intake, energy output in urine, methane and milk ($P < 0.05$ or 0.01), although the difference in faecal energy output, heat production or energy retention was not significant between the two breeds. There was no significant difference between the 2 breeds in the efficiency of ME utilisation for lactation (k_l), or energy partitioning between milk (milk energy/ME intake) and body tissue (retained energy/ME intake).

Table 1 Energy intake and output and the efficiency of energy utilisation for Holstein and Jersey-Holstein dairy cows

Concentrate level (kg/kg DM)	Holstein		Jersey-Holstein		s.e.	Breed	Conc. L	Significance ¹	
	0.30	0.70	0.30	0.70					
GE intake (MJ/d)	269	306	280	328	6.6	*	***		
Faecal energy output (MJ/d)	61	68	62	74	2.2		***		
Urine energy output (MJ/d)	10	11	10	12	0.4	*	**		
Methane energy output (MJ/d)	18	18	20	20	0.6	**			
Heat production (MJ/d)	116	134	119	134	2.6		***		
Milk energy output (MJ/d)	56	67	62	72	2.2	*	***		
Retained energy (MJ/d)	8	8	7	17	3.8				
Milk energy/ME intake	0.32	0.33	0.34	0.33	0.011				
Retained energy/ME intake	0.04	0.03	0.03	0.07	0.019				
Efficiency of ME use for lactation	0.57	0.54	0.57	0.58	0.014				

¹ There was no significant interaction on any parameter between cow breed and concentrate level (Conc. L)

* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$

Conclusions Results of the present study indicates similar energetic efficiency and energy partitioning between milk and body tissue between Jersey-Holstein and Holstein dairy cows with two planes of nutrition.

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Evaluation of progeny from Limousin bulls with either a Top 1% or Bottom 1% Beef Value

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Introduction In a previous study at Harper Adams University College (Marsh *et al.*, 2007) the progeny from Limousin bulls with either a Top 1% Beef Value (LM44) or Top 10% Beef Value (LM30) were reared through to slaughter on a cereal beef system. The calves sired by the Top 1% Beef Value bull recorded significantly higher daily liveweight gains, daily carcass gain, slaughter weights, carcass weights, improved conformation scores and carcass values. The objective of this trial was to evaluate the performance of Limousin cross Holstein-Friesian bull and heifer calves sired by bulls with either a Top 1% or Bottom 1% Beef Value.

Materials and methods In June 2004 Holstein-Friesian dairy cows were inseminated with semen from the Limousin bulls; Killerton Travis (Top 1% Beef Value of LM44) and Nouvelle Tonic (Bottom 1% Beef Value of LM0). The calves were reared through to slaughter on a cereal beef system with 30 bulls and heifers per sire. The cattle were housed in straw-bedded pens and fed a 140g CP/kg cereal mix *ad libitum*. The bulls were selected for slaughter at MLC fat class 3 and heifers at fat class 4L. The data was analysed using ANOVA.

Results The calves sired by the Top 1% Beef Value sire recorded significantly higher ($P < 0.01$) daily carcass gains, killing out percentage with reduced number of days to slaughter. They also recorded significantly higher ($P < 0.05$) DLWG's, carcass weights, improved conformation scores and carcass values compared to calves sired by the Bottom 1% Beef Value sire. 'Beef Value' is an assessment of the economic value of an animal's genetic merit. The theoretical difference between the progeny from the sires should have been £22. In this study the difference in carcass value was £32.20 per calf and the earlier slaughter was estimated to be worth £13.63 thus giving a net benefit of £45.83 exceeding the predicted value by £23.83.

Table 1 Animal Performance (bulls & heifers)

	Top 1%	Bottom 1%	s.e.d	Sig
Gestation (days)	286.1	284.5	1.35	NS
Calving ease ¹	1.46	1.31	0.156	NS
Birth weight (kg)	48.2	47.1	0.87	NS
Slaughter weight (kg)	534.2	530.4	7.70	NS
Days to slaughter	396.2	413.3	5.66	**
DLWG (kg)	1.227	1.168	0.0223	*
Carcass wt (kg)	296.1	283.6	5.20	*
Kill out (g/kg)	554	533	0.48	**
Carcass daily gain (kg)	0.690	0.632	0.0189	**
Conformation class ²	3.85	3.42	0.167	*
Fat class ²	3.69	3.72	0.150	NS
Carcass price (£/kg)	1.95	1.92	0.012	NS
Carcass value (£)	575.4	543.2	11.93	**

¹ Calving Ease: 1 = unassisted, 5 = caesarian, ² EUROP carcass classification: Conformation: P+=1 and E=7, Fat class: 1=1 and 5H=7.

Table 2 Animal Performance (bulls versus heifers)

	Top 1%		Bottom 1%		s.e.d
	Bulls	Heifers	Bulls	Heifers	
Slaughter weight (kg)	584.5	483.9	574.1	486.8	12.99
Days to slaughter	404.7	387.8	429.8	396.7	8.22
DLWG (kg)	1.326	1.129	1.221	1.115	0.0367
Carcass wt (kg)	330.1	262.0	312.1	255.2	8.8
Kill out (g/kg)	564	541	544	524	0.65
Conformation class	3.92	3.77	3.54	3.31	0.187
Carcass daily gain (kg)	0.759	0.620	0.673	0.590	0.0219

There were no significant sire by sex interactions.

Conclusions The performance of bulls and heifers sired by the Top 1% Beef Value bull exceeded the recognised targets for cereal fed Continental cross beef cattle. In this study genetic merit of a breeding sire presented in terms of a selection index called 'Beef Value' was closely positively correlated with the actual financial returns achieved by the bulls' progeny in a finishing system. In fact the economic benefit was greater than that suggested by the Beef Value. Therefore it is clear that beef producers aiming to prosper in a decoupled beef industry should exploit the benefits offered to them through breed improvement and the genetic information available (EBVs and selection indexes) for recorded bulls.

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Study of the genetic links between two Belgian horse breeds by the study of their genetic variability

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Introduction In Belgium, sport horse breeding is organized by the Belgian Warmblood Horse (BWP) studbook and the Belgian Sport Horse (sBs) studbook. Genetic links between both populations have been suspected because BWP and sBs studbooks had concomitant development after the Second World War, because they are geographically close, and because they have the same selection objectives (model, jumping and dressage ability). The conservation of genetic variability between breeds is important, even in selected populations, in order to allow a long term management of the entire genetic resources available for future improvements. The purpose of this study was to evaluate the genetic links between BWP and sBs populations by the study of their comparative genetic variability. Some studies already dealt with a similar problem (Moureaux *et al.*, 1996; Valera *et al.*, 2005).

Materials and methods Pedigree data from BWP and sBs studbooks until 2006 were merged, and a unique identification number was given to horses registered in both of them. Evolution of the breeds of origin of registered horses was described. The genetic variability of BWP and sBs populations was described by computing several parameters with specific software: (1) The probability of gene origin was described by the number of founders (horses with unknown parents), by the effective number of founders (f_e : minimum number of founders explaining the complete genetic diversity). The 1000 main ancestors (horses contributing largely to the current genetic variability without necessarily being a founder) were identified with their genetic contributions to the reference population, and an effective number of ancestors (f_a) was also computed. The contribution of different breeds was described according to ancestor contributions. (2) The average inbreeding coefficient (F) in BWP and sBs populations was computed as the probability that two alleles of one random gene of an individual were identical by descent. The average coancestry coefficient (J) between BWP and sBs populations was computed as the probability that one allele for a random gene was identical by descent for two horses chosen in each population. (3) The genetic distances (d) between BWP and sBs populations, defined as $-\log_e [J_{(BWP-sBs)} (J_{(BWP)}J_{(sBs)})^{-1/2}]$, were computed.

Results In 2006, there were near 250,000 horses registered in BWP studbook and 55,000 in sBs studbook. 1 horse in 30 was registered in both studbooks. After 1995, mares were mainly registered in Belgian studbooks at birth but more than 60% of the approved stallions still came from foreign studbooks. The results associated to the genetic variability are presented in Table 1. The main genetic influences came from French Saddle, German breeds and English Thoroughbred. On the 1000 ancestors found for females, 192 were common to both breeds, which was equivalent to a genetic contribution of 47%. The results of the genetic distances between sub-groups of the populations are presented in Figure 1.

Table 1 Parameters of genetic variability computed for BWP and sBs populations

	BWP	sBs	BWP-sBs
Founders ¹	14,876	6,130	
f_e ¹	714	657	
Ancestors ^{1,2}	603	558	
f_a ¹	143	133	
F (%)	0.85	0.93	
J (%)	0.60	0.67	0.53

¹ of the female reference population (2001-2006)

² cumulated contribution of 80%

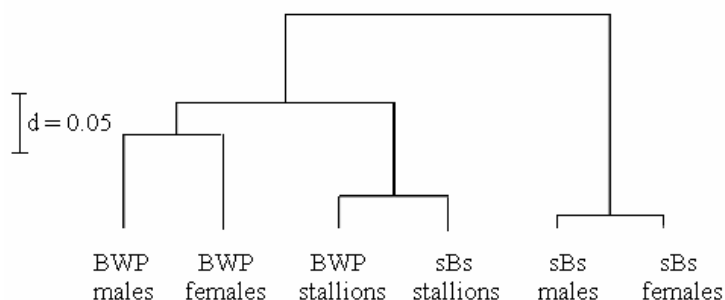


Figure 1 Genetic distances among subgroups of BWP and sBs populations

Conclusions The study revealed that both studbooks showed a great genetic variability; the main reason was the open policy of registrations of the studbooks, mainly with foreign stallions. However there was an unbalanced use of ancestors. According to the number of ancestors, the genetic bases of BWP and sBs were wide but half of the current genetic pool was common to both breeds. This was due to extensive international exchanges of breeding stock helped by the improvement of artificial reproduction techniques. Nevertheless, relationship and inbreeding coefficients were low. There were clear genetic links between BWP and sBs populations through the approved stallions, but they still formed two distinct genetic groups.

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Changes in genetic merit of silkworm lines over eight generations

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Introduction The main objective of silkworm breeding programs is collecting the appropriate genes in the populations to improve productive performance lines (Jayaswal *et al.*, 2000). The general aim of selection in animal breeding is to obtain new generation of animals, which under future economic conditions of production system are more efficient than the present generation. In selection index programmes, top animals are selected based on their multiple-trait genetic-economic value named genetic merit. In this method, genetic trend of important traits is directly based on their weights in economic efficiency of production system to acquire maximum improvement in economic merit (Shadparvar *et al.*, 2005). The objective of this study was to study changes in the genetic merit of 6 silkworm lines over 8 generations.

Materials and methods This research was carried out on six commercial silkworm lines including 110, 107, 101433, 153, 154 and Y. The data used were approximately 16000 records for cocoon weight (CW), cocoon shell weight (CSW) and cocoon shell percentage (CSP) which were obtained from eight generations (24 full sib families reared in each generation) in each line. After estimating (co)variance components by REML method, the additive genetic values were predicted using BLUP. The economic values of traits were derived using a deterministic model developed by data simulation method. Total genetic merit (H) in six lines were defined based on estimated economic values (v) and additive genetic values (g) for the three traits as follows: $H = v_{cw}g_{cw} + v_{csw}g_{csw} + v_{csp}g_{csp}$. Then, the average total genetic merit of each silkworm line was estimated.

Results The average total genetic merit of the silkworm lines over eight generations is shown in Figure 1. The highest improvement in total genetic merit was seen in the 153 line and the lowest one was in Y and 110 lines. Total genetic merit increased slightly up to third generation. There was a rapid rise in these parameters in fourth generation and after fifth generation average genetic merit rose moderately. In all generations, total genetic merit of the 153 line increased more sharply. For other lines, there was a relatively similar trend in the parameter. Previous studies were showed the 153 line had high heritability for cocoon weight, cocoon shell weight and cocoon shell percentage. Thus, it was expected that this line would have the highest rate of improvement in average total genetic merit in comparison to other lines.

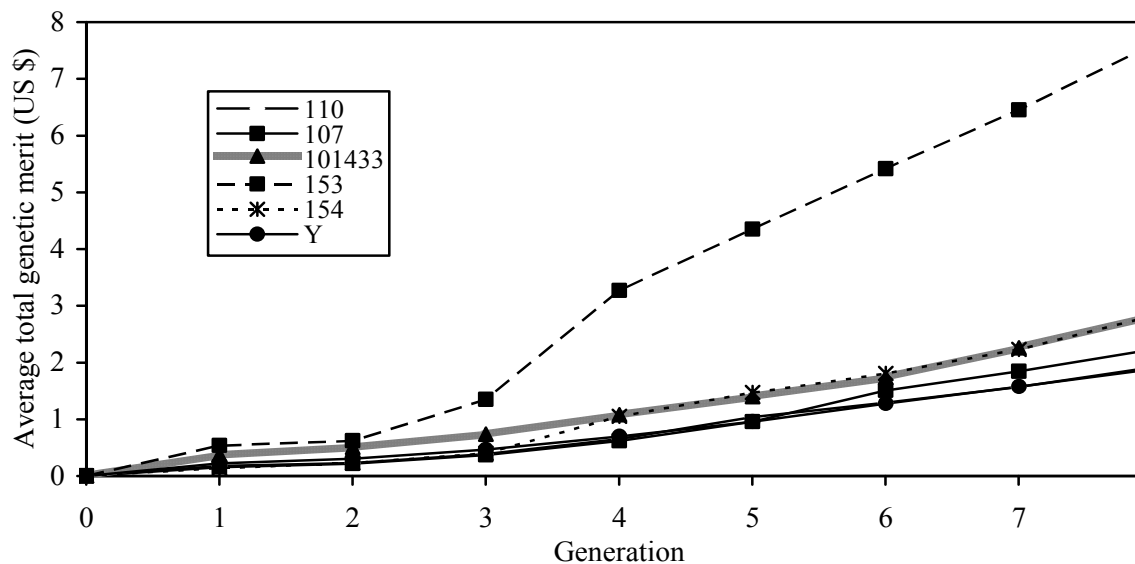


Figure 1 Average total genetic merit (per moth) in silkworm lines

Conclusions The results obtained demonstrated that trend of genetic merit changes in commercial silkworm lines is different. In fact, genetic and economic responses to selection vary among them. Up to third generation, lines showed small changes in their average genetic merit, because inadequate records were collected and genetic parameters of the populations were estimated with low accuracy. It caused genetic gains of traits to decrease. After fifth generation, the rate of change declined because of decrease in genetic variability. Knowledge of actual economic response to selection can help to silkworm breeder to use high economically efficient lines in hybridization program to maximize cocoon producers' profitability in the future economic conditions.

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Polymorphism in exon 3 of Bovine Leptin Gene in Iranian Holstein cattle

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Introduction Leptin is a 16-kDa polypeptide hormone synthesized and secreted predominantly by adipose tissue. It functions to regulate body weight, food intake, energy expenditure, reproductive and immune functions. Leptin treatment of animals has been shown to cause a decrease in food intake, body weight loss, fat deposit weight loss and increase in energy metabolism. Therefore leptin not only causes reduced food intake, but the potential body weight losses are enhanced due to an increased metabolic rate (Houseknecht *et al.* 1998). The gene encoding Leptin was mapped to Bovine chromosome 4 and it consists of 3 exons and 2 introns of which only two exons are translated into protein. The coding region of the leptin gene (501 nucleotides in length) is contained in exon 2 and 3, which are separated by intron of approximately 2 kb. Leptin is considered as a candidate gene for milk performance related traits in cattle. Several polymorphism in this gene have been found (Liefers *et al.* 2002). In exon three, A59V polymorphism, causes an amino acid change from Alanine to valine. These amino acids both belong to the group of aliphatic amino acids, but Valine is more hydrophobic. The aim of this study was to analyse the frequency of the A59V Polymorphism in exon 3 of Leptin Gene in Iranian Holstein cattle.

Materials and methods Blood was collected from 255 Holstein cattle belonging to four different herd managements in Isfahan province. Genomic DNA extracted from whole blood. Genotypes of A59V Polymorphism were identified with PCR-RFLP technique. Amplified region is located in exon three of leptin gene. The genomic bovine leptin sequences, which consist of three exons, were obtained from GeneBank (Accession number U50365). The polymerase chain reaction was used to amplify the 331 bp DNA fragments from genomic DNA. The PCR reaction contained 100 ng of genomic DNA, 0.3 μ M of each primer, 1.5 mM MgCl₂, 200 μ M dNTP, 10mM Tris HCl, 50 mM KCl and 1 U *Taq*-polymerase in total volume of 20 μ L. Sequences of primers that were used in PCR were reported previously by Haegeman *et al.* (2000). The sequence of the forward and reverse primers, respectively were: 5'-GGG AAG GGC AGA AAG ATA G-3' and 5'-TGG CGA ACT GTT GAG GAT C-3'. Conditions for PCR were 94°C for 2 min, followed by 35 cycles of 94°C for 30 s, 55°C for 1 min, and 72°C for 30 s. Followed by final extension for at for 15 min 72°C. Digestion of PCR product of 331 bp with with 5 U of *HphI* (Fermentas) in 20 μ L of reaction volume at 37°C for 8 h and analysed on 8% non-denatured polyacrylamide gel. Allele A in the A59V polymorphism was the allele not digested by restriction enzyme, allele B was the restriction enzyme-digested PCR product. Digestion revealed 3 genotypes, AA (331 bp), AB (331, 311, and 20 bp), and BB (311 and 20 bp). The PCR reaction result in an artefact band of 600 bp which did not interfere, as it was not digested by the enzyme. The allele and genotype frequencies of A59V polymorphism were examined for deviation from Hardy-Weinberg equilibrium by χ^2 test.

Results Genotype and allele frequencies of the A59V polymorphism are listed in Table 1. Genotype frequencies in all herd were 0.588, 0.388 and 0.024 for AA, AB and BB, respectively and gene frequencies were 0.782 and 0.218 for allele A and B, respectively. allelic frequency analysis have shown that frequency of the A allele was ranged from 0.759 to 0.824 and the frequency of the B allele was calculated to be ranged from 0.176 to 0.241 in all herds. In 2th herd we did not find any BB genotype. The genotype frequencies were distributed according to Hardy-Weinberg equilibrium proportions in every four herd but were not in all herd ($p < 0.05$).

Table 1 Genotype and allele frequencies of the A59V polymorphism

Herd	N	Genotype			Allele	
		AA	AB	BB	A	B
1	58	0.552	0.414	0.034	0.759	0.241
2	54	0.648	0.352	0.000	0.824	0.176
3	40	0.650	0.325	0.025	0.813	0.188
4	103	0.553	0.417	0.029	0.762	0.238
total	255	0.588	0.388	0.024	0.782	0.218

Conclusions Our findings for A59V polymorphism in bovine leptin gene are similar to those of Hanna Kulig (2005) who reported A and B allele frequencies of 0.760 and 0.240, respectively. Liefers *et al.* (2002) found a frequency of 0.747 for the A allele and of 0.254 for the B allele. These results show that allele B has lower frequency in all study. This polymorphism could be further evaluated for marker assisted selection.

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Polymorphism of insulin-like growth factor I in Iranian Holstein cows

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Introduction Molecular biology techniques are of help in genetic improvement since they permit the identification, mapping and analysis of polymorphisms of genes encoding proteins that act on metabolic pathways involved in economically interesting traits. The somatotrophic axis, which essentially consists of growth hormone releasing hormone (GHRH), growth hormone (GH), insulin like growth factors I and II (IGF-I and IGF-II), and their associated binding proteins and receptors (GHRHR, GHR, IGF-IR and IGF-IIR), plays a key role in the metabolism and physiology of mammalian growth (Curi *et al.*,2005). Polymorphic sites in genes involved in the mediation of growth factors are logical candidates to study for possible associations with livestock production traits. The objective of this study was to estimate the allele and genotype frequencies of the IGF-I/*Sna*BI gene polymorphism in Iranian Holstein cows and their relations with performance traits.

Materials and methods Blood was collected from 250 Holstein cows from 4 different herd managements in Isfahan province. Genomic DNA was extracted from whole blood. Genotypes of IGF-I were identified with PCR-RFLP technique. Polymerase chain reaction was used to amplify the 249 bp DNA fragments from genomic DNA. The PCR reaction contained 25-50 ng of genomic DNA, 10 pmol of each primer, 2µl 10X PCR buffer, 1.5 mM MgCl₂, 200 µm dNTP and 1 unit *Taq*-polymerase in total volume of 20 µL. Sequences of primers that were used in PCR were reported previously by Ge *et al.* (2001). Sequences of IGFR and IGFF were 5'-CCTTACCCGTATGAAAGGAATATACGT-3' and 5'-ATTACAAAGCTGCCTGCC-3', respectively. Conditions for PCR were 97°C for 2 min, followed by 31 cycles of 94°C for 60s, 58°C for 45s, and 72°C for 60s. The final step was at 72°C for 5 min. Eight micro litres of PCR products was digested with 5 units of *Sna*BI (Fermentas Co.) in 20 µL of reaction volume at 37°C for 4 h for RFLP of IGF-I gene. The digested products plus 2 µL of loading dye were run on 2% Agarose gel for 2h and the genotypes were determined under UV lights. The effect of IGF-I genotypes on the milk production traits were analysed using GLM procedure of SAS and the least square means of the genotypes were compared. The linear model used to fit the quantitative variables included, in addition to the genotype effect, as follows: $Y_{ijk} = \mu + I_i + H_j + b_1(X_{ijk}-X) + b_2(Z_{ijk}-Z) + b_3(W_{ijk}-W) + e_{ijk}$, where Y_{ijk} = production trait, μ = overall mean, I_i = fixed effect of the *i*th genotype (AA, AB, BB), H_j = fixed effect of the *j*th herd (1, 2, 3, 4), X_{ijk} is days of milking, b_1 is the linear regression coefficient of days of milking, Z_{ijk} is a vector of dry days, b_2 is the linear regression coefficient of dry days, W_{ijk} is open days, b_3 is the linear regression coefficient of open days, and e_{ijk} is the random error.

Results The A and B alleles of the IGF-I gene were identified based on the amplification of a 249 bp fragment located in the regulatory region of the gene 512 bp upstream of the initiation codon, followed by digestion with the restriction enzyme *Sna*BI in agreement with Ge *et al.* (2001). Two genetic variants (A and B) of the IGF-I polymorphism were observed in this experiment. Genotype AA was characterised by the presence of two restriction fragments of 226 and 23 bp, while genotype BB was determined by the presence of a single 249bp fragment. AB individuals presented three fragments of 249, 226, and 23 bp. The AB and AA genotypes were the most (0.583-0.661) and lowest (0.083-0.192) frequents in all herds respectively. The frequency of BB genotype was from 0.201 to 0.333. However allelic frequency analysis has shown that frequency of the A allele was ranged from 0.375 to 0.495. Then, the frequency of B was calculated to be ranged from 0.504 to 0.625. The population was not in Hardy Weinberg equilibrium (Table 1) and AB genotype affected fat and protein percent significantly ($p < 0.1$ and $p < 0.05$ respectively) (Table 2).

Table 1 Frequencies of genotypes and alleles of the IGF-I gene in Iranian Holstein cow.

Herd	n	Genotypes			Alleles	
		AA	AB	BB	A	B
1 st	56	0.089	0.661	0.250	0.420	0.580
2 nd	54	0.130	0.630	0.241	0.444	0.556
3 rd	36	0.083	0.583	0.333	0.375	0.625
4 th	104	0.192	0.605	0.201	0.495	0.504
Ave./ Total	250	0.142	0.630	0.227	0.457	0.542
Expected Hardy-Weinberg Equilibrium		0.208	0.495	0.293	$X^2=17.2845^*$	
		849	388	764		

* $X^2=0.0002$, df=2

Table 2 Least square means and standard errors of the milk yield traits obtained for the genotypes of the IGF-I/*Sna*BI polymorphisms.

Polymorphism Genotype		Fat percent	Protein percent
		LSMean ±SE	LSMean ±SE
IGF-I/ <i>Sna</i> BI	AA	2.548 ±0.060 ^a	2.609±0.092 ^a
	AB	2.669±0.029 ^b	2.833±0.044 ^b
	BB	2.568±0.043 ^a	2.702±0.066 ^a

a,b: significantly different least square means ($p < 0.1$).

Conclusions Because IGF-I genotype frequencies was affected by selection on milk production traits, the population was not in Hardy Weinberg equilibrium. The polymorphism we have identified at the IGF-I locus could be a potential genetic marker for fat and protein percent.

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Polymorphism of the Growth Hormone Gene in Iranian Holstein Cows

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Introduction Selection in dairy breeding is basically aimed at milk production and composition of milk (Kovacs *et al.*, 2006). Bovine growth hormone gene is an excellent candidate for linkage relationships with quantitative trait loci (QTL). A polymorphic region in exon V of the bovine GH gene results in a nucleotide change related to the occurrence of variants with leucine (*Leu*) or valine (*Val*) at amino acid position 127 of the protein. This polymorphism was found to be associated with milk production traits (Lucy *et al.*, 1993). The aim of the present study was to explore the frequency of GH/*AluI* alleles in Iranian Holstein cows and look for relationships between the bGH gene and milk production traits.

Materials and methods In total, blood samples from 257 cows of the Holstein breed were collected via mammary vein and were saved in tubes containing EDTA. Genomic DNA was isolated from whole blood by Miller protocol (Miller *et al.*, 1988) and, the genotypes were identified with PCR-RFLP technique. The primers (F: 5'-CCGTGTCTATGAGAAGC-3', and R: 5'-TTCTTGAGCAGCGCGT-3') flanked a 428 bp fragment, including intron IV and parts of exons IV and V. The PCR was performed in a reaction volume of 10 μ l, 1 mM MgCl₂, 200 μ M each dNTP, 0.2 μ M primers, 10 x PCR buffer, 0.5 unit *TaqI* DNA polymerase (Sinagen Co.) and 25-50 ng genomic DNA. Thirty amplification cycles included: 94°C, 30 s; 60°C, 1 min; and 72°C, 30 s. PCR products (The 428-bp fragment of the GH gene) were digested in a total volume of 20 μ l, containing the whole amount of PCR products, 5 units of *AluI* enzyme (Promega Co.) for 4 h at 37°C. Digested products were scored for expected fragments of 265, 96, 51 and 16 bp for the L variant and 265, 147 and 16 bp for the V variant. The effect of GH genotypes on the milk production traits of Holstein cows were analysed using GLM procedure:

$$Y_{ijk} = \mu + ST_i + Herd_j + b_1(X_{ijk} - \bar{X}) + b_2(Z_{ijk} - \bar{Z}) + b_3(W_{ijk} - \bar{W}) + e_{ijk}$$

where: Y_{ij} is the phenotypic record of the trait, μ is the overall mean, ST_i is the fixed effect of bST genotype ($i = 1, \dots, 3$), $Herd_j$ refers to the fixed effect of the herd ($j = 1, \dots, 4$), X_{ijk} is days of milk, b_1 is the linear regression coefficient of days of milk, Z_{ijk} is a vector of dry days, b_2 is the linear regression coefficient of dry days, W_{ijk} is open days, b_3 is the linear regression coefficient of open days, and e_{ijk} is the random error.

Table 1 Frequencies of genotypes and alleles of the bGH gene in Iranian Holstein cows

Herd	n	Genotypes			Alleles	
		LL	LV	VV	L	V
1	105	56	45	4	0.7476	0.2524
2	39	18	18	3	0.6923	0.3076
3	58	35	18	5	0.7586	0.2414
4	55	30	22	3	0.7454	0.2546
Ave./Total	257	139	103	15	0.7412	0.2588
Expected	Hardy-Weinberg	0.54	0.38	0.06	Chi-Square* = 0.516	
Equilibrium		9	3	7		

$X^2 = 0.772$ (df = 2)

Results Digestion of PCR products produced 4, 5, and 3 RFLP bands for homozygous leucine 127, heterozygous leucine/valine and homozygous valine 127 animals, respectively. All three possible genotypes were detected. The LL genotype was the most frequent in all herds. The population was in Hardy-Weinberg equilibrium (Table 1). The effect of GH locus was not significant on milk production traits.

Conclusion The population is in Hardy-Weinberg equilibrium; therefore selection for milk yield did not differ in GH alleles frequencies. On the basis of the statistical analysis, it can be concluded that highly strong relationship between GH genotype and milk production traits could not be detected. Hence it could be concluded in order to find suitable bGH allele as a marker for increasing production traits, both GH genotype and GH concentration in plasma should be considered. It can be possible to establish or deny the presence of association between GH genotypes and milk production with a larger sample in order to have a higher number of rare genotype, VV.

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Ruminal degradability parameters of corn grain processed using various methods

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Introduction Grain sources and processing have been discussed for many years. Most feedlots select their grain source based on cost of the grain plus its processing. A variety of processing methods have been devised that vary in cost and effectiveness. The primary goal of processing is to increase energy (starch) availability. In addition, processing may destroy mycotoxins and improve mixing characteristics to improve bunk management and thereby enhance animal performance (Owens *et al.*, 1997). Mechanical and chemical alterations during processing increase surface area exposure and improve ruminal, intestinal and total tract starch digestibility of seed grains (Huntington, 1997; Owens *et al.*, 1997). Unprocessed corn can be effectively fed to ruminants because the pericarp of the kernel is extensively damaged by chewing (Beauchemin *et al.*, 1993). So the objective of this study was to investigate effects of processing method and size of corn ground particles on ruminal degradation parameters.

Materials and methods 1) Whole corn, 2) Dry rolled 3) Wet rolled 4) Ground corn >8 mesh, 5) Ground corn >10 mesh, 6) Ground corn >35 mesh 7) Ground corn <35 mesh 8) Whole NaoH treated corn were sampled weighing approximately 4.0 g as fed and were placed in 8×12-cm bags, which were made of Dacron polyester (pore size: 46 µm) and incubated at times 0, 2, 4, 6, 12, 24 and 36 in three ruminally castrated Brown Swiss heifers which were fed to meet maintenance requirements (NRC 2001). Procedures for *in situ* bag technique were totally those recommended by Cruywagen (2006). Bags were hand washed by rinsing until the wash water from the bags ran clear. Bag washing took 1 min to remove remaining ruminal particles adhering to the bags and any remaining filterable or soluble material. Bags at each time period were dried at 57 °C for 24 h and were weighed to determine DM remaining. Weights of all bags were blank corrected using empty bag weights receiving the same treatments as the feed bags to correct for residue adhering to bags. Dry matter disappearance was calculated as the percentage difference between the amount of sample and the final DM remaining after rumen incubation and rinsing. Dry weights were not corrected for microbial contamination, because contribution of microbial contaminants to dry weight of residue is minimal, and dry weights were blank corrected. Degradability parameters (A: soluble fraction%, B: slowly digestible fraction%, C: fractional rate of disappearance per hour) and effective degradability% (ED) determined using Chen software (Fitcurve, Version 6) and compared between process methods using proc GLM procedure of SAS 9.1. Means compared using duncan test (P<0.05).

Results Table 1 show that NaoH treatment of corn can effectively improve the ruminal degradation parameters (A and ED) in compare with whole corn (P<0.05) and NaoH improved the parameters. Wet and dry rolled corn didn't show any considerable differentiations in most ruminal degradation parameters except B and C (P<0.05). Size of ground corn doesn't have any critical role in rumen degradation and all parameters are equal between 8, 10 and 35 mesh except corn under 35 mesh which is significantly higher (P<0.05). Dry and wet rolled corn had increased ED in compare with ground, whole or NaoH treated ones (P<0.05).

Table 1 Effect of corn processing method and particle size on some ruminal degradability parameters

Treatments	Corn								SEM
	Whole	NaoH treated	Wet rolled	Dry rolled	mesh 8<	mesh 10<	mesh 35<	mesh 35>	
A %	4.2 ^c	11.2 ^d	19.1 ^b	21.1 ^b	8.7 ^{ed}	8.9 ^{cd}	12.2 ^c	25.4 ^a	0.830
B %	93.1 ^c	88.8 ^{bc}	80.9 ^a	78.9 ^c	91.3 ^{bc}	91.1 ^{bc}	87.8 ^{ab}	73.5 ^c	8.084
C /hour	0.0003 ^{bc}	0.005 ^b	0.006 ^c	0.02 ^b	0.002 ^c	0.002 ^c	0.002 ^c	0.097 ^a	0.004
ED % ¹	9.0 ^c	31.7 ^b	58.4 ^a	58.0 ^a	42.5 ^b	44.3 ^b	47.1 ^b	73.2 ^a	3.585

1- Effective degradability – means in each row with different subscripts are significantly different (P<0.05)

Conclusion Results showed that reducing corn size and using NaoH instead of whole corn or using wet rolling can improve the situation of corn effective degradability in rumen and affect its ruminal degradability parameters.

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Silage characteristics of a halophyte tropical forage growth in Iran

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Introduction Increasing population and lack of water resources brings the attention of investigators to types of halophytes growing in salty soil and water areas in order to guarantee forage for animals. *Kochia Scoparia* is a potentially valuable forage plant on arid and semiarid lands, because it yields DE and protein comparable to alfalfa with about half the water requirement (Finley and Sherrod, 1971). However, its value is decreased by poor utilisation of nutrients (Cohen *et al.*, 1989). It has been reported that adding *kochia scoparia* forage to sheep ration does not affect the nutrient digestibility, but increasing different levels of *kochia* in cattle and sheep rations cause decreased retention of nitrogen in the body of these animals (Sherrod, 1973). This study is focused on introducing *Kochia scoparia* as a native forage in Iran and investigates the characteristics of it when ensiled.

Materials and methods *Kochia scoparia* was harvested from deserts around Sabzevar city in northeast of Iran (Khorasan-razavi) using Sabzaver Jihad-e agriculture branch facilities. *Kochia* was chopped at 8 ± 1.25 cm and completely mixed with different amounts of molasses (0, 5 and 10 percent) via a factorial experiment based on a completely randomized design and according to the weight fed, then ensiled in experimental silos (polymer buckets, 40 cm height and 30 cm diameter). 24 experimental silos were divided between treatments (0, 5 and 10 percent molasses) and opened in 1, 2, 3 and 21 days after ensiling. Samples were taken and pH was determined immediately using pH meter (Greisinger electronics, GPHR 1400 A). Fresh samples were mixed with distiller water (1:1) and then pH was determined. Another sample was frozen for future chemical analysis. DM, OM, ADF, Ca and CP were determined using standard methods. Data were analyzed using SAS 9.1 and means were compared using Duncan test ($P<0.05$).

Results Data showed that silage pH decreased with increasing molasses levels ($P<0.0001$). Also pH was decreased with a low rate, up to 21 days after ensiling, compared with corn silage that reduced to pH 4.5 after only 2 days. DM content increased with molasses addition but reduced with days after ensiling ($P<0.0001$). OM increased with addition of molasses ($P<0.006$) but reduced with days after ensiling ($P<0.0015$). OM will increase due to the addition of molasses as it has more DM content than *Kochia* but decreases with time due to usage by silage micro flora. Ca content of silage was not affected with day or treatments.

Table 1 Chemical composition of ensiled *Kochia scoparia* with different amounts of molasses in various days

Items	% Molasses			SEM	Pvalue		
	0	5	10		Treat	Day	Treat×Day
pH	6.46 ^a	4.98 ^b	4.70 ^c	0.024	**	**	**
DM (%)	38.90	40.12	43.60	0.250	ns	**	**
OM (%)	87.10 ^b	87.50 ^b	88.10 ^a	0.160	**	**	**
Ca (mg/ml)	0.76	0.76	0.83	0.052	ns	ns	ns
	Days						
	1	2	3	21			
pH	6.22 ^a	5.52 ^b	5.21 ^c	4.57 ^d	0.027		
DM (%)	39.53 ^b	38.40 ^c	38.12 ^c	37.08 ^c	0.290		
OM (%)	88.04 ^a	87.81 ^a	86.92 ^b	---	0.160		
Ca (mg/ml)	0.732	0.745	0.858	---	0.052		

- Means with different characters are significantly difference. ns: non-significant **: $P<0.01$

Conclusion It seems that addition of molasses to *Kochia Scoparia* silage can increase DM and OM content and decrease pH level in a short time so the silage characteristics of *Kochia Scoparia* can be improved via molasses addition.

Acknowledgments This study was conducted at Education Center of Jihad-e Agriculture in Mashhad city.

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Lactic acid bacteria as a corn silage additive and its effects on fattening performance of Brown Swiss male calves

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Introduction The primary goal of making silage is to maximize preservation of original nutrients in the forage crop for feeding at a later date. Unfortunately, fermentation in the silo is an uncontrolled process that usually leads to less nutrient preservation. In order to assist in the fermentation process, various silage additives have been used to improve the nutrient and energy recovery in silage, often with subsequent improvements in animal performance. One method which has been successfully used is inoculating silage with a specially selected lactic acid bacterium (LAB) which is known to process good silage making qualities. This study is focused on some practical aspects of the fermentation process and the uses of silage additives containing lactic acid bacteria (ECOSYL AND BIOTAL).

Materials and methods Nineteen Brown Swiss bull calves with 166.6 ± 46.57 Kg average live weight at a Brown Swiss herd (Animal Science Department Farms, Education Center of Jihad-e Agriculture) were selected to test the effective use of silage additives containing lactic acid bacteria (ECOSYL). The calves are classified and randomly allocated to three experimental treatments in a completely randomized design with unequal replicates include: 1) whole corn silage without additive (Control) 2) whole corn silage processed with additive containing lactic acid bacteria (BIOTAL) 3) whole corn silage processed with additive containing lactic acid bacteria (ECOSYL). Length of experiment was 110 days. Feed was made as TMR and fed ad libitum to calves. Dietary for all treatments were equalised. Only the corn silage was the source of dietary variation between treatments. Rations were adjusted to NRC (1996) recommendations. Intake was measured daily during design period. Faeces were collected in days 105-110 and used to measure digestibility. Blood and rumen liquid samples were obtained in 61 and 92 d 2 hours after morning feeding. Weighing was done at ten o'clock each 2 weeks. Data were analyzed using the GLM procedure of SAS 9.1 and means were compared using Duncan test ($P < 0.05$).

Results Data showed that using LAB had no significant effect on DMI. Although there was a numerical reduction which can be because of adding LAB and more organic acid production and it had an adverse effect on DMI (Steen *et al*, 1998; Wright *et al*, 2000). Daily weight gain was not affected by treatments just a slight numerical reduction because of DMI reduction. Feed to gain wasn't affected by using additives in corn silage preservation because there was a parallel decrease in both feed and gain. Biotal and Ecosyl in compare with control hadn't showed any difference at physical characteristics too. Results showed that DM, OM, and CP digestibility was not affected by these additives but NDF digestibility when calves were fed corn silage inoculated with Ecosyl was significantly reduced in comparison with Biotal and control ($P < 0.05$). In most of the researches which had done before there was an improvement in nutrient's digestibility (Henderson *et al*, 1990; Anderson *et al*, 1989). Rumen pH and N-NH₃ was equal between calves at various treatments. Blood metabolites (Urea and Glucose) were not affected by LAB. Faecal score was significantly reduced with LAB compared with control at day 105 of the experiment ($P < 0.05$). Rectal temperature in Ecosyl was significantly lower than others in day 60 ($P < 0.05$) showing a better health status of these calves because LAB is a Probiotic.

Table 1 Effect of corn silage inoculated with different lactic acid bacteria on some male Brown Swiss calves parameters

Items	Treatment			SEM
	Control	Biotal	Ecosyl	
Dry matter intake (Kg/day)	9.21	8.45	7.65	0.305
Daily weight gain (Kg)	1.41	1.33	1.29	0.045
Feed to gain	6.50	6.43	6.13	0.244
Total Hip height gain (Cm)	7.57	6.92	6.00	0.447
Total Stomach size gain (Cm)	13.57	15.33	12.58	1.265
Total Heart girth gain (Cm)	15.86	17.33	14.17	1.326
NDF ¹ Digestibility (%)	47.46 ^a	43.16 ^a	33.84 ^b	1.761
Faecal Score ² Day 105	3.00 ^a	2.49 ^{ab}	2.50 ^b	0.102
Rectal temperature (°C) Day 60	38.89 ^{ab}	38.95 ^a	38.67 ^b	0.049

Items with different letter in each row have significant difference ($P < 0.05$)

1- Neutral detergent fiber 2- Based on a 1-3 system

Conclusion It seems that LAB in this experiment had no significant effects on Brown Swiss male calves' performance despite reducing NDF digestibility. LAB did reduce faecal score.

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Ruminal degradability of neutral detergent fibre and neutral detergent insoluble nitrogen of feed supplements

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Introduction In ruminant feeding, the prediction of metabolizable protein requires accurate estimates of feed protein degradation in the rumen. Some common feed protein sources have a high concentration of neutral detergent insoluble nitrogen (N-NDF) whose degradation may be dependent on neutral detergent fibre (NDF) degradation and, consequently, on rumen cellulolytic activity. This may lead to an underestimation of the undegraded protein that reaches the small intestine, particularly when animals are fed high starch diets. The objective of this study was to verify if there is a relationship between the ruminal degradability of the NDF and N-NDF of eight feed supplements currently used in ruminant feeding with the *in sacco* technique.

Materials and methods Twelve samples of eight feed supplements (dried distillers' maize, corn gluten feed, coconut meal, palm kernel meals, sunflower meal, groundnut meal, cottonseed meal and dehydrated lucerne) were used in this study. Samples (1 mm) were analysed for dry matter (DM) and crude protein (CP) according to AOAC (1990) and NDF and N-NDF according to Van Soest *et al.* (1991). Samples (4 mm) were incubated *in sacco* (46 µm pore size; Ørskov *et al.*, 1980) for 2, 4, 8, 16, 24, 48, 72 and 96 h. The daily ration of the 3 cannulated rams consisted of meadow hay, beet pulp and soya bean meal making 50:35:15 on DM basis. Residues from individual animals were analysed for N-NDF and NDF. Degradation data were analysed using the exponential model: $p=a+b(1-\exp(-ct))$ where p is the degradation, t is the time of incubation, a is the soluble and/or rapidly degradable fraction, b is the potential degradable fraction and c is the constant rate of degradation. One-way ANOVA analysis was used to compare degradation constants between feeds.

Results Chemical composition of feed supplements (Table 1) is in good agreement with current tabulated values. The N-NDF fraction represents a large proportion of the CP (higher than 50%) in palm kernel meals and coconut meals. Differences in the degradation constants between feed supplements for NDF and N-NDF were found (Table 1). As expected, the soluble and/or rapidly degradable fraction was low for both NDF and N-NDF, representing the losses of small particles from the bags. Higher ($P<0.05$) degradation rates were observed for sunflower meal for both NDF and N-NDF. The correlation between degradation rate for NDF and N-NDF was significant ($r=0.814$; $n=12$; $P<0.001$). These data show that the N-NDF degradation rate is lower than the degradation rate of NDF for all the feeds studied, confirming that the degradation of N-NDF depends on the NDF degradation. This probably indicates that a large amount of feed protein may be affected by the lower ruminal cellulolytic activity when animals are fed high starch diets.

Table 1 Chemical composition and degradation constants of NDF and N-NDF

Feed supplements	Chemical composition			Degradation constants					
				NDF			N-NDF		
	CP	NDF	N-NDF	a	b	c	a	b	c
	(%DM)			(%)			(%/h)		
Coconut meal 1	22.5	58.7	2.72	2.7 ^b	58.0 ^b	3.52 ^{ab}	4.5 ^{cd}	48.7 ^{cd}	2.00 ^a
Coconut meal 2	21.3	62.5	2.51	2.3 ^b	61.7 ^b	3.88 ^b	3.3 ^{bc}	45.2 ^c	2.15 ^a
Palm kernel meal 1	16.3	72.2	1.71	1.1 ^a	57.4 ^b	3.11 ^a	6.2 ^e	31.1 ^a	2.63 ^b
Palm kernel meal 2	17.5	73.2	1.81	0.8 ^a	59.6 ^b	4.02 ^b	2.0 ^a	30.2 ^a	2.91 ^{bc}
Sunflower meal	30.0	45.6	0.91	2.5 ^b	57.7 ^b	6.77 ^d	5.2 ^d	60.2 ^e	4.65 ^e
Groundnut meal 1	52.8	24.2	1.38	2.2 ^b	62.4 ^b	5.86 ^c	5.5 ^d	58.5 ^e	3.61 ^d
Groundnut meal 2	43.9	31.6	0.99	2.2 ^b	68.0 ^{cd}	5.71 ^c	3.0 ^{ab}	57.6 ^e	3.58 ^d
Cottonseed meal	43.1	26.8	0.65	3.0 ^b	65.3 ^{bc}	5.65 ^c	2.8 ^{ab}	50.9 ^{cd}	3.17 ^c
Dehydrated lucerne	13.7	46.9	0.33	3.6 ^c	59.3 ^b	4.97 ^c	2.0 ^a	43.3 ^{bc}	2.81 ^{bc}
Dried distilled maize 1	26.9	47.2	2.14	3.3 ^c	71.7 ^d	3.18 ^a	9.7 ^g	39.3 ^b	2.91 ^{bc}
Dried distilled maize 2	28.8	48.2	2.21	3.7 ^c	67.8 ^c	3.21 ^a	7.8 ^f	29.0 ^a	2.76 ^b
Corn gluten feed	21.9	36.7	0.86	4.1 ^c	45.9 ^a	4.17 ^b	9.7 ^g	35.1 ^a	3.12 ^c
Probability				0.007	<0.001	0.002	0.033	<0.001	0.005

Values in the same column with different letter are significant different ($P<0.05$).

Conclusions These results show that the N-NDF degradation constants varied greatly between feed supplements. Our data also indicates that the degradation of the N-NDF fraction seems to be dependent of on the NDF degradation.

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Methods of barley grain processing and its ruminal disappearance *in situ*

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Introduction Barley is a dominant feed grain in Iran, western North America, and Europe. Barley processing is imperative to maximize its utilisation by feedlot and dairy cattle (Yang *et al*, 2000). Whole grain with an intact pericarp is largely or entirely resistant to digestion by ruminants because whole kernels are resistant to bacterial attachment (Beauchemin *et al*, 1994) in the rumen. In addition to pericarp, barley grain is surrounded by a fibrous hull, which is of low digestibility. Barley kernels are not severely damaged by chewing. Consequently, considerable whole barley kernels are excreted in feces if whole barley kernels are fed. So the objective of this study was to investigate effects of processing on ruminal degradability of barley and trend of ruminal digestion of ground barley in different sizes.

Materials and methods Three ruminally castrated Brown Swiss heifers were fed a forage based diet to meet maintenance requirements (NRC 2001). Barley samples were rolled, grounded and passed through 8, 10 and 35 mesh sieves, wholly processed or used wholly. Treatments include: 1) Whole barley, 2) Dry rolled 3) Wet rolled 4) Ground barley >8 mesh, 5) Ground barley >10 mesh, 6) Ground barley >35 mesh 7) Ground barley <35 mesh and 8) Whole NaOH treated barley. Samples weighed approximately 4.0g as fed, were placed in 8×12cm bags, which were made of Dacron polyester (pore size: 46 µm) and incubated at times 0, 2, 4, 6, 12, 24 and 36. Procedures for *in situ* bag technique were totally those recommended by Cruywagen (2006). Bags were hand washed by rinsing until the wash water from the bags ran clear. Bag washing took 1 min to remove remaining ruminal particles adhering to the bags and any remaining filterable or soluble material. Bags at each time period were dried at 57 °C for 24 h and were weighed to determine DM remaining. Weights of all bags were blank corrected using empty bag weights receiving the same treatments as the feed bags to correct for residue adhering to bags. Dry matter disappearance was calculated as the percentage difference between the amount of sample and the final DM remaining after rumen incubation and rinsing. Dry weights were not corrected for microbial contamination, because contribution of microbial contaminants to dry weight of residue is minimal, and dry weights were blank corrected. Degradability parameters (A: soluble fraction%, B: slowly digestible fraction%, C: fractional rate of disappearance per hour) and effective degradability% (ED) determined using Chen software (Fitcurve, Version 6) and compared between process methods using proc GLM procedure of SAS 9.1. Means compared using duncan test (P<0.05).

Results As can be seen in Table 1 whole barley have low potential of rumen degradability (P<0.05). Using NaOH as a processing method increases its degradability in the rumen (P<0.05). Dry rolled barley has more ruminal degradation potential than Wet rolled barley and the rate of degradability is larger (P<0.05). Taking a look at Table 1 show that by decreasing the size of barley when passing from sieves 8 to 35 mesh the A fraction gradually increased and B begins to decrease (P<0.05). Also the effective degradability increases by reducing size of barley grain (P<0.05).

Table 1 Degradability parameters of processed barley and its different particle sizes

Treatments	Barley								SEM
	Whole	NaOH treated	Wet rolled	Dry rolled	mesh 8<	mesh 10<	mesh 35<	mesh 35>	
A %	7.3 ^{cd}	14.3 ^c	15.4 ^c	16.0 ^b	8.0 ^e	8.7 ^{de}	13.2 ^c	32.4 ^a	1.24
B %	5.4 ^d	85.7 ^a	67.1 ^{bc}	72.8 ^{bc}	89.5 ^b	91.3 ^b	78.9 ^{bc}	57.1 ^c	9.07
C /hour	0.137 ^d	0.003 ^e	0.216 ^a	0.286 ^a	0.076 ^d	0.140 ^{bc}	0.227 ^{ab}	0.110 ^c	0.013
ED % ¹	11.8 ^c	49.4 ^d	75.1 ^c	83.4 ^{ab}	76.2 ^c	86.7 ^a	83.8 ^{ab}	80.7 ^b	1.42

1- Effective degradability – means in each row with different subscripts are significantly different (P<0.05)

Conclusion With the understanding that the dacron bag technique measures disappearance and not necessarily degradation of material from the bags in the rumen then, it seems that wet rolling of barley can be used instead of dry rolling to decrease barley ruminal disappearance rate and size of ground barley plays an important role in its disappearance behaviour (Controlled rate of degradation) in the rumen. NaOH can be used to some extent increasing barley rumen disappearance but almost low effective disappearance makes it hard to recommend and it seems that more researches is needed deciding if NaOH can be used as suit grain processing method.

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Effects of gamma irradiation on protein degradation characteristics of pea

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Introduction Production of field peas has been promoted in most areas of the world due to their high level of protein content as a substitute for soybean meal. The majority of the field peas are grown under contract for human consumption. Field peas must meet strict quality guidelines to enter the human market. The peas that are not eligible for human consumption are then available for livestock feed. This feed cannot be used judiciously in feeding ruminants because of their high degradability in the rumen. In highly productive ruminants, peas may not provide adequate amounts of escape protein which will necessitate supplementation of the diet with high escape protein sources. Gamma irradiation of soybean meal resulted in denaturation of proteins, increasing hydrophobicity of the protein surface and decreasing degradation of crude protein (CP) in the rumen (Shawrang *et al.*, 2007). The objective of this study was to evaluate the effects of γ -irradiation on crude protein degradation parameters and, on the fate of true proteins of pea in the rumen.

Materials and methods Four pea samples (500 g each, 25% moisture content) were subjected to γ -irradiation at doses of 0, 25, 50 and 75 kGy using cobalt-60 irradiator at room temperature. Duplicate nylon bags of untreated and γ -irradiated pea were suspended into the rumen of four non-lactating Holstein cows for 0, 2, 4, 6, 8, 12, 16, 24, 36 and 48 h. The *in situ* incubation of nylon bags was the same as that described by Shawrang *et al.* (2007). Proteins of untreated and treated bag residues were fractionated by gel electrophoresis according to Laemeli (1970). Digestibility of CP was measured using mobile nylon bag technique (De Boer *et al.*, 1987). Crude protein was measured according to AOAC (1995). Degradation kinetics was calculated using the model of Ørskov and McDonald (1979). Data were analyzed as a randomized complete block design, with animals as blocks using the GLM procedure of SAS (1996).

Results As shown in Table 1, γ -irradiation decreased ($P < 0.05$) washout fraction and increased ($P < 0.05$) the potentially degradable fraction of CP. The degradation rate of the latter fraction decreased with this treatment. As a consequence, γ -irradiation decreased significantly the effective CP degradability of pea. Gamma irradiation had no effect ($P > 0.05$) on the intestinal mobile bag digestibility of ruminally undegraded CP. From gel analyses, pea proteins were composed of two major components; globulins (7S and 11S fractions) and albumins. In untreated pea, the albumin and globulin 7S subunits disappeared more rapidly than globulin 11S. Albumin subunits of γ -irradiation at doses of 0, 25, 50 and 75 kGy were degraded completely within 2, 2, 4 and 6 h and for globulin 7S within 4, 4, 8 and 12 h of incubation, respectively. The globulin 11S subunits of untreated pea were degraded slowly and represented a large proportion of the protein remaining after 12 h of incubation. In γ -irradiated pea at doses of 25, 50 and 75 kGy, globulin 11S were not completely degraded after 12, 24 and 48 h of incubation. There were cross-linked products of the protein molecules that could not penetrate the running gel.

Table 1 The crude protein degradation kinetics of untreated and gamma irradiated pea

Items	Washout fraction	Potentially degradable fraction	Degradation rate (per h)	ED [†] 0.02	ED 0.05	ED 0.08	Intestinal digestibility
Untreated pea	0.365 ^a	0.618 ^c	0.082 ^a	0.861 ^a	0.748 ^a	0.677 ^a	0.925
25 kGy g-irradiated pea	0.352 ^a	0.622 ^c	0.076 ^b	0.844 ^{ab}	0.727 ^{ab}	0.655 ^a	0.921
50 kGy g-irradiated pea	0.307 ^b	0.659 ^b	0.070 ^c	0.819 ^{bc}	0.691 ^{bc}	0.614 ^b	0.936
75 kGy g-irradiated pea	0.239 ^c	0.689 ^a	0.066 ^c	0.767 ^c	0.632 ^c	0.550 ^c	0.929
S.E.M	0.0105	0.0193	0.0030	0.0254	0.0276	0.0227	0.0173

S.E.M., standard error of mean; Means in the same column with different superscripts differ ($P < 0.05$).

[†] ED: Effective degradation calculated with a solid outflow rate from the rumen of 0.02, 0.05 and 0.08 per h.

Conclusion Pea proteins appeared to be effectively protected from ruminal degradation by γ -irradiation at doses higher than 25 kGy, without adverse effect on intestinal digestibility of CP.

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***In vitro* gas measuring technique for evaluating the nutritional quality of Iranian safflower seed and comparing them with other oil seeds**

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Introduction Safflower seed (*Corthamus tinctorius*) was recognized to contain the highest concentration of linoleic acid among all oilseeds (Dubois *et al.*, 2007), but research in this field is a few and almost non for Iranian safflower seed. The use of *in vitro* gas method based on syringes appears to be most convenient in developing countries where resources may be limited in recent years (Makkar, 2004). The Objective of this study was to compare nutritive value of two varieties of safflower seed (IL-111(I) and Native (N)) cultivated in Isfahan, Iran to each other and to the other oilseeds.

Materials and methods Samples of several varieties of oilseeds including two varieties of Iranian safflower, cottonseed, soybean and canola provided by Isfahan branch of oilseed research and development co. were used in this experiment. Chemical composition of oilseeds shown in Table 1. In this experiment gas measuring technique described by Blummel (1994) (modified method of original method of Menke *et al.*, 1979) was used to compare nutritive value of oilseeds (Getachew *et al.*, 1998). Ruminally fistulated (435 kg) Holstein steer were used for obtained rumen liquor sample. The steer fed a TMR twice daily at 0900 and 1600 h. TMR included 2.7 kg of alfalfa hay, 1.75 kg of corn silage and 2.25 kg of concentrate (barley 63.5, cottonseed meal 5.8, beet pulp 17.3, wheat bran 10.0, limestone 1.0, salt 0.4, vitamin-mineral supplement 0.5, and urea 1.5%) on a DM basis. Liquid rumen fraction were collected before the morning feeding, placed in an insulated plastic container, sealed immediately and transported to the laboratory. Media solution mixed with rumen liquor in a ratio of 1:2(v/v) (rumen liquor: media solution) and immediately bubbled with CO₂ for maintaining anaerobic condition. Triplicate samples of about 500 mg DM of each grinded oilseed were placed into three 100 ml glass syringes (Haberle Labortechnik, Germany) and 30 ml of mixture of rumen liquor and media solution were added to these, and excess gas released. Three syringes containing only 30 ml of the media solution and rumen liquor were used as blanks. The syringes were vertically positioned in a water bath at 39 °C. All syringes were gently shaken 30 min after the start of the incubation and, thereafter, four times each day. Gas production was recorded at 0, 2, 4, 8, 12, 24, 48 and 72 h of incubation. The gas production parameters measured by NEYWAY Excel (1995) and data were analyzed as a complete randomized design using the general linear means procedure of SAS (1997). The treatment means were statistically compared with the duncan method. Differences between treatments were statistically significant if $P < 0.001$.

Results Gas production parameters of five experimental oilseeds are shown in Table 2. There was significant variation between oilseeds in potential gas production (a+b) and there was no significant variation between constant rates of gas production during incubation (c) in oil seeds ($p < 0.001$). The safflower seeds (I and N) and cottonseed produced a similar amount of gas.

Table 1 Chemical composition of experimental oil seeds(% DM)

	DM	CP	EE	NDF	ADF	Ash
Soybean	89.9	20.5	40.5	17.8	11.6	4.6
Canola	91.8	19.2	41.9	24	16.7	5.1
Cottonseed	90.1	23.5	19.3	50	40.1	4.2
Safflower(I)	95.3	19.44	22.7	51.8	45.2	3.07
Safflower(N)	93.5	17.96	31	46	39.5	2.5

Table 2 Gas production parameters

	a+b(ml)	c(h ⁻¹)
Soybean	123.85 ^a	0.046 ^a
Canola	87.3 ^b	0.053 ^a
Cottonseed	43.95 ^c	0.046 ^a
Safflower(I)	37.5 ^c	0.055 ^a
Safflower(N)	38.35 ^c	0.048 ^a
SEM	11.6	0.000009
<i>P</i>	0.0001	n.s.

Conclusions This study showed that there were no significant differences between potential gas production of Iranian safflower seeds (N) or (I) and cottonseed, as well as their chemical composition and nutritional quality. These results confirm the results of Mahdavi and Nikkhah (2004) for gas production parameter of cottonseed. Results from this study indicated that there are some resemblances between safflower and cottonseed. However, additional investigations for its composition, which may have negative impact on ruminant performances, are needed.

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The effect of exogenous fibrolytic enzymes on lucerne hay nutritive value using *in vitro* gas production and *in situ* techniques

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Introduction Fibrolytic enzymes are typically cellulases that degrade cellulose or xylanases that act on hemicellulose (Beauchemin *et al.*, 1995). Enzymes applied to forages immediately before incubation enhance the digestion of dry matter (DM) and neutral detergent fiber (NDF) suggesting that fibrolytic enzymes applied at feeding can improve digestion in ruminants. The objective of this study was to determine the effect of fibrolytic enzyme on the nutritive value of lucerne hay using *in vitro* gas production (GP) and *in situ* techniques.

Materials and methods Rumen fluid was collected from two fistulated sheep, fed twice daily a diet containing digestible energy (DE; 14.01 MJ/kg DM) and crude protein (CP; 160 g/kg DM) consisting of forage (400 g/kg) plus commercial sheep concentrate (600 g/kg), 2 hour after morning feeding and strained through four layers of cheesecloth. Lucerne hay was dried using a forced-air oven at 96 °C for 48 h, ground to pass through a 2-mm screen. The enzyme mixture (Natuzyne, Bioproton Pty. Ltd. Co.) contained phytase, beta glucanase, alpha amylase, cellulase, hemicellulase, pectinase, xylanase. Gas production was measured by Fedorak and Hrudý (1983) method. Approximately 300 mg of dried lucerne hay was weighted and placed into 50 ml capacity serum bottles then enzyme mixture add in liquid form (diluted with tap water 1:20) at rate of 3, 6, 12 g/kg DM and incubated in 20 ml of buffered rumen fluid (ratio of buffer to rumen fluid was 2 : 1) for 48 h. The GP profiles, in triplicate, fitted with equation of $Y = A(1 - e^{-ct})$ where Y is the volume of GP (ml/g DM) at time t , A is GP from soluble and insoluble fraction, c is the fractional rate of GP (/h) and t is time (h). The data at the different times was analysed using completely randomized design by the GLM procedure of SAS Institute Inc (2002).

Results The *in vitro* GP and *in situ* disappearance results are shown in Tables 1 and 2 respectively. There was a significant differences between treatment in incubation times ($P < 0.001$). Gas production volumes and DM disappearance of treated lucerne with enzyme (3, 6, 12 g/kg DM) were higher than control (water added to lucerne). The GP volumes and DM disappearance in incubation times had significant differences ($P < 0.001$). The moderate concentration of enzyme (6 g/kg DM) result in higher GP volume and also the disappearance of DM of this treatment were higher than the other treatments in *in vitro* and *in situ* that can be due to the negative effect of enzyme at high levels. The *in situ* potentially degradable fraction (b) and *in vitro* (a+b) at 6 g/kg was greater than the other treatments (Table 1 and 2).

Table 1 Gas production parameters of treatments *

	treatments				S.E.M
	Control	3 g/kg DM	6 g/kg DM	12 g/kg DM	
A (ml/g DM)	217.8 ^d	278.8 ^b	279.5 ^a	260.3 ^c	0.001
c (/h)	0.11 ^a	0.087 ^c	0.093 ^b	0.082 ^d	0.003
Effective gas production (ml)	187.17 ^d	231.54 ^b	234.75 ^a	213.61 ^c	0.001

* The means within a rows without common letter differ ($P < 0.001$).

Table 2 *In situ* dry matter disappearance parameters *

	treatments				S.E.M
	Control	3 g/kg DM	6 g/kg DM	12 g/kg DM	
a	26.17 ^d	26.8 ^b	27.21 ^a	26.48 ^c	0.001
b	28.07 ^d	30.06 ^c	35.12 ^a	30.68 ^b	0.001
c (/h)	0.0388 ^b	0.0343 ^c	0.0638 ^a	0.0387 ^b	0.001
ED	44.7 ^d	54.8 ^a	53.9 ^b	46.7 ^c	0.001
Outflow rate	0.02	0.02	0.02	0.02	

* The means within a rows without common letter differ ($P < 0.001$).

Conclusions The current study indicated that exogenous enzymes have the potential to increase forage degradation in the rumen. The ruminal fermentation characteristics of treated alfalfa with Natuzyne showed slight changes compared to untreated lucerne hay using the GP and *in situ* techniques.

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Effects of ethanol treatments on degradation kinetics of crude protein and rumen undegraded protein of canola meal

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Introduction Canola meal (CM) is a commonly used protein supplement for ruminants, the proteins of which are extensively degraded in the rumen. Attempts to decrease the rate and extent of ruminal degradation of canola meal proteins have used physical and chemical treatments (Sadeghi and Shawrang, 2006). However, most of these treatments adversely affect the protein digestibility of the final product in the small intestine. Ethanol treatment appears to be superior in that it alters protein degradability without reducing its intestinal digestibility. No information is available concerning effects of ethanol treatment on ruminal crude protein (CP) degradation and type of true proteins of canola meal that leave the rumen undegraded. Therefore, the objectives were to investigate the effects of ethanol treatment at levels of 500, 600 or 700 ml/l on protein degradability and intestinal digestibility of CM, and to monitor the fate of true proteins of treated CM in the rumen.

Materials and methods Four canola meal samples (600 g each) were soaked (1:2 w/v) in water (control), 500, 600 or 700 ml/l ethanol solutions for 2 h. After excess liquids were decanted, treated samples were freeze-dried and allowed to equilibrate at ambient temperature for 2 h prior to sealing in plastic bags. Duplicate nylon bags of untreated or treated CM were suspended into the rumen of four non-lactating Holstein cows for 0, 4, 6, 8, 12, 16, 24, 36 and 48 h. Degradation kinetics was calculated using the model of Ørskov and McDonald (1979). Proteins of untreated and treated bag residues were fractionated by gel electrophoresis according to Laemeli (1970). Digestibility of CP was measured using mobile nylon bag technique (De Boer *et al.*, 1987). Crude protein was measured according to AOAC (1995). Data were analysed as a randomised complete block design, with animals as blocks using the GLM procedure of SAS (1996).

Results Ethanol treatments applied to canola meal decreased linearly ($P < 0.05$) the washout fraction (a) of CP (Table 1). The effects of ethanol treatment on the potentially degradable fraction (b), the estimated rate (c) of CP degradation, and effective degradable protein were quadratic ($P < 0.001$) and linear ($P < 0.05$). The lowest estimated rate of CP degradation and the calculated effective degradable protein was observed when CM was treated with 600 ml/l ethanol solution. Extending the ethanol concentration over 600 ml/l to CM was considered excessive. Intestinal digestibility of ruminally undegraded CP were affected quadratically ($P < 0.001$) and linearly ($P < 0.05$) with ethanol treatments. At concentration of 500, 600 and 700 ml/l, digestibility of CP increased by 4, 10 and 9%, respectively. Electrophoretic analyses of untreated CM protein residues revealed that napin subunits (albumin fraction) were degraded completely at zero incubation time, and for the 500, 600 and 700 ml/l ethanol treated CM, they were degraded after 2, 4 and 4 h of incubation times, respectively. In untreated CM, cruciferin subunits (globulin fraction) were degraded at 12 h of incubation, but in treated CM were not degraded after 48 h of incubation.

Table 1 Ruminal CP degradation and intestinal protein digestibility of untreated and ethanol treated CM

Parameters	Untreated canola meal	Ethanol treated canola meal			S.E.M	Contrasts		
		500 ml/l	600 ml/l	700 ml/l		L	Q	C
a	0.254	0.171	0.139	0.152	0.0083	***	NS	NS
b	0.706	0.776	0.821	0.811	0.0221	*	***	NS
$a + b$	0.961	0.947	0.960	0.963	0.0160	***	NS	NS
c (/h)	0.064	0.047	0.039	0.041	0.0051	*	***	*
Effective rumen degradation at different rumen outflow rate								
0.02 /h	0.793	0.715	0.682	0.697	0.0224	**	***	NS
0.05 /h	0.652	0.547	0.498	0.517	0.0206	*	**	NS
0.08 /h	0.569	0.458	0.408	0.426	0.0169	*	**	NS
Intestinal digestibility	0.753	0.782	0.808	0.783	0.0190	*	***	NS

S.E.M, standard error of the means; L, linear contrast; Q, quadratic contrast; C, cubic contrast. Significance: NS, not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. a , immediately-soluble fraction, b , the potentially degradable fraction and c the rate of degradation.

Conclusions It was concluded that treatment of canola meal with 600 ml/l ethanol solution had the greatest potential to increase rumen undegradable protein.

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The effect of oil content of various feed protein sources on *in situ* and *in vitro* ruminal and post-ruminal protein disappearance

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Introduction *In situ* rumen incubation and post-rumen mobile nylon bag technique, and *in vitro* enzymatic procedure are currently used for estimating ruminal and post-ruminal disappearance of feed protein (Danesh Mesgaran and Stern, 2005). The objectives of the present study was to determine the ruminal and post-ruminal protein disappearances of various feed protein sources containing different oil concentrations, using *in situ* rumen incubation and post-rumen mobile nylon bag technique, and an *in vitro* enzymatic procedure.

Materials and methods Feed protein sources containing different oil concentrations, produced in various Iranian oil industries, were cottonseed meal (5, 40 and 80 g oil/kg dry matter (DM); CS5, CS40 and CS80, respectively), rapeseed meal (30 and 70 g oil/kg DM; RS30 and RS70, respectively) and soyabean meal (6 and 50 g oil/kg DM; SB6 and SB50, respectively). Ruminal and post-ruminal protein disappearance of the samples were evaluated *in vivo* using the *in situ* disappearance and mobile nylon bag techniques and *in vitro* using an enzymatic procedure (Danesh Mesgaran and Stern, 2005). Approximately 1.2 g of sample DM was placed in a polyester bag (3 cm × 6 cm; pore size of 52 µm; 16 bags/feed) and incubated in the rumen of four Holstein steers (395 ± 13 kg), fitted with rumen and T-shaped intestinal cannulae, for 12 h (assumed out flow rate of 0.08/h). After removal from the rumen, half of the bags were inserted into the small intestine via the intestinal cannulae, then removed from the voided faeces, washed and dried (64 °C, 48 h). For the *in vitro* enzymatic procedure, 1 g of each sample was weighed into polyester bags, pore size of 22 µm (n= 6), placed into borate-phosphate buffer and incubated for 1 h at 39 °C in a shaking Incubator. Then, 400 ml of protease (Sigma, P-5147) solution (1980 units protease in 400 ml of borate-phosphate buffer) were added and after 4 h incubation (39 °C), the bags were removed and rinsed. Three bags per sample were dried (64 °C, 48 h) and the remaining bags were placed in a pepsin solution [1.6 g of pepsin (Sigma P-7000) in 800 ml of a 0.1 N HCl]. Bags were incubated for 1 h at 39 °C. Then, 40 ml of 1 N NaOH and 1 litre of the pancreatin solution [6 g of pancreatin (Sigma, P-7545) and 68 g of KH₂PO₄ in 1 litre of distilled water] was added, then incubated for 24 h at 39 °C. After incubation, bags were removed and washed and dried (64 °C, 48 h). Nitrogen concentration of each pre-incubated and incubated sample was determined using the Kjeldhal method. Data were analysed using the general linear models procedure of SAS (1999) with the statistical model of Y= overall mean+ block effect of procedure+ feed effect within block effect+ residual error.

Results The ruminal and post-ruminal protein disappearance values of the various feed protein sources are shown in Table 1. The procedures had a significant effect ($P < 0.05$) on the rumen and post-rumen protein disappearance of the feeds evaluated in the present study. Results showed a significant ($P < 0.05$) effect of feed protein sources with different oil concentration on ruminal and post-ruminal protein disappearance.

Table 1 Rumen and post-rumen protein disappearance values of the various protein sources studied containing different oil concentrations using the *in situ* and enzymatic *in vitro* procedures

Protein sources	Rumen CP disappearance						Post-rumen disappearance of rumen-undegraded CP					
	Procedure		Feed effect*		Procedure effect*		Procedure		Feed effect		Procedure effect	
	<i>In situ</i>	<i>In vitro</i>	s.e.m.	P	s.e.m.	P	<i>In situ</i>	<i>In vitro</i>	s.e.m.	P	s.e.m.	P
CS5	0.74	0.81	0.04	<0.05	0.03	<0.05	0.91	0.81	0.04	<0.05	0.3	<0.05
CS40	0.46	0.71					0.83	0.73				
CS80	0.54	0.70					0.71	0.54				
RS30	0.89	0.85					0.70	0.88				
RS70	0.84	0.80					0.64	0.80				
SB6	0.84	0.91					0.85	0.96				
SB50	0.61	0.88					0.84	0.94				

* When the difference between means is greater than two times the s.e.m., it is considered as significant ($P < 0.05$).

Conclusions There was a considerable variation in rumen and post-rumen protein disappearance of the feed protein sources evaluated by *in situ* and *in vitro* methods. Samples of cottonseed meal with low oil concentration had higher rumen and post-rumen protein disappearance values compared with CS40 and CS80. Therefore, modifications of the *in situ* and *in vitro* methods for estimating the digestibility of high oil containing feed protein sources need to be developed.

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***In vitro* gas production parameters of high fat sunflower meal treated with formaldehyde, sodium hydroxide or exogenous enzyme**

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Introduction Gas production technique is a useful procedure to assess digestible value of the ruminant feeds. The feeding value of the sunflower meal (SFM) depends on the oil extraction process, variety of sunflower and the proportion of the hulls removed during the extraction. Formaldehyde decreases protein degradability and NaOH (Chen *et al.*, 2007) and exogenous enzymes (Eun and Beauchemin, 2007) increase digestibility. The objective of this study was to investigate the effect of formaldehyde, sodium hydroxide or exogenous enzymes on the gas production parameters of sunflower meal containing high fat (165 g fat /kg DM) during *in vitro* fermentation.

Materials and methods The samples were: untreated SFM (USFM), NaOH treated SFM (40 g/kg DM, NSF), exogenous enzyme treated SFM (5 g/kg DM, ESF); the enzyme mixture composition was Cellulase, Xylanase, Betagluconase, Alpha amylase, Pectinase, Phytase, Protease and Lipase as 0.03, 6.6, 10, 0.7, 0.7, 0.07, 0.5 and 3 MU/kg, respectively; Bioproton Pty. Ltd. Co.) and formaldehyde treated SFM (30 g/kg DM, FSF). Crude protein, NDF and lignin content of the samples were 278, 400 and 83 g/kg DM, respectively. About 500±10 mg of oven dried and milled sample (1.0 mm screen) was incubated with 35 ml buffered rumen fluid (Rumen fluid was collected from two 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 100 ml glass syringes, according to the method of Menke and Steingass (1988). All samples were incubated in triplicate (one run) with three syringes containing only incubation medium (blank) and gas production from the sample was corrected for the blank. Gas production was measured at 2, 4, 6, 8, 12, 24, 48, 72 and 96 h. 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 is the gas production rate constant for B , t is the incubation time (h) and Y is the gas produced at time t . Ammonia-N ($\text{NH}_3\text{-N}$) concentration (mg/dl) was determined in supernatant samples at the end of the incubation time by macro Kjeltec System Tecator (Büchi 1030, Sweden). *In vitro* digestibility of organic matter (OMD, g/kg OM) and metabolizable energy (ME, MJ/kg DM) of samples were calculated by the equation of Menke and Steingass (1988). Short chain fatty acid concentration (SCFA, μmol) was measured by the equation as proposed by Getachew *et al.* (1999). Data of gas production, ME, OMD, $\text{NH}_3\text{-N}$ and SCFA were subjected to analysis as a completely randomized design using the General Linear Model (GLM) procedure of SAS (1990). Duncan's multiple range test was used to compare treatment means at $P < 0.01$.

Results *In vitro* gas production parameters [B and C], ME, OMD, $\text{NH}_3\text{-N}$ and SCFA of the samples are shown in Table 1. All items were significantly influenced by the treatment. Gas production parameters of NSF and ESF were significantly higher than FSF ($P < 0.01$). Formaldehyde resulted in decreased OMD, ME and SCFA compared with NSF and ESF. $\text{NH}_3\text{-N}$ concentration was decreased when sunflower meal was treated with formaldehyde. The highest SCFA was recorded for ESF compared with the other samples.

Table 1 *In vitro* gas production parameters, $\text{NH}_3\text{-N}$ concentration, ME, OMD and SCFA of high fat sunflower meal treated with formaldehyde, sodium hydroxide or exogenous enzyme

Item	Treatments*				s.e.m	p-value
	USFM	NSFM	ESFM	FSFM		
B (ml)	167 ^c	185 ^b	196.9 ^a	116.9 ^d	0.8	0.01
C (ml/h)	0.08 ^c	0.1 ^b	0.18 ^a	0.07 ^d	0.002	0.01
$\text{NH}_3\text{-N}$ (mg/dl)	40.99 ^a	36.22 ^c	39.19 ^b	32.47 ^d	0.4	0.01
ME (MJ/kg DM)	29.38 ^c	35.75 ^b	39.2 ^a	29.37 ^c	0.1	0.01
OMD (g/kg OM)	185.9 ^d	207.5 ^b	210.2 ^a	189.7 ^c	0.1	0.01
SCFA (μmol)	1.20 ^c	1.53 ^b	1.63 ^a	0.62 ^d	0.001	0.01

*USFM (untreated sunflower meal); NSF (40 g/kg DM NaOH treated sunflower meal); ESF (5 g/kg DM exogenous enzyme treated sunflower meal); FSF (30 g/kg DM formaldehyde treated sunflower meal); B : Gas production from the fermentable fraction; C : Rate constant of gas production; OMD: Organic matter digestibility; ME: Metabolizable energy; SCFA: Short chain fatty acids; s.e.m: Standard error of mean, Means with different letters within samples differed ($P < 0.01$)

Conclusions It was concluded that *in vitro* gas production parameters, OMD, ME and SCFA of NaOH and enzyme treated SFM were improved compared with USFM and FSF. In contrast, formaldehyde treated SFM caused a decrease in gas production parameters, $\text{NH}_3\text{-N}$ and SCFA concentrations. Therefore, based on the present data, formaldehyde is not recommended to treat SFM, when much fermentation is a goal of the feeding strategy.

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Relationship between *in situ* degradation kinetics and *in vitro* gas production fermentation using different mathematical models

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Introduction The development of alternative *in vitro* techniques, such as the gas production technique, has led to the introduction of several mathematical models to describe and interpret the fermentation characteristics of feedstuffs. While there are enough data to validate each gas production model regarding its potential to estimate *in situ* degradation parameters, few studies have been conducted to compare models. The aim of this study was to analyse the relationship between the *in situ* degradation characteristics of several feedstuffs and the gas production parameters using different mathematical models.

Materials and methods Fifteen feedstuffs were evaluated. All feed samples were dried at 70°C and ground to pass a 1mm screen before use. All samples were analysed in duplicate for ash, crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), starch and sugar. Measurements of *in situ* OM were performed in 3 rumen fistulated dairy cows using the nylon bag technique (Ørskov and McDonald, 1979). Residues after different incubation periods were fitted to first-order degradation in order to calculate constant rate of degradation (k_d). Fermentable organic matter (FOM) was calculated using *in situ* characteristics, assuming a rumen passage rate of 6%h⁻¹. Gas production incubations were performed in fully automated equipment in duplicate in a single run with rumen fluid collected from 2 non-lactating cows, 2 h after the morning feeding. Data were recorded every 20 minutes. Gas curves were fitted by iteration to the mono-phasic Exponential (Ørskov and McDonald 1979), Logistic (Schofield *et al.*, 1994), Gompertz (Lavrencic *et al.*, 1997) and Groot (Groot *et al.*, 1996) models. The *in situ* parameters were estimated from chemical and gas production variables using multiple regression analysis. Independent variables with $P < 0.05$ were included in the regression models.

Results CP in the feedstuffs ranged from 66 g.kg⁻¹ DM in citrus pulp to 559 g.kg⁻¹ DM in soybean meal, starch ranged from 2 g.kg⁻¹ DM in pressed beat pulp to 183 g.kg⁻¹ DM in maize gluten feed, sugars ranged from 2 g.kg⁻¹ DM in brewery grains to 272 g.kg⁻¹ DM in citrus pulp and NDF ranged from 82 g.kg⁻¹ DM in maize to 590 g.kg⁻¹ DM in palm kernel expeller.

Table 1 Relationship between *in situ* parameters and chemical and gas production variables.

Model	Predicted ¹	Variable ²	R ²	P	RSD
Exponential	U	V _F , kn, CP, ADF	0.97	<0.001	1.7
	FOM	V _F , sugars, NDF	0.46	0.0025	7.9
Logistic	U	V _F , CP, ADF	0.94	<0.001	2.3
	kd	Sn, CP	0.79	<0.001	1.2
	FOM	µm, NDF	0.71	<0.001	7.1
Gompertz	U	V _F , CP, ADF	0.94	<0.001	2.4
	kd	Cg, A, kn, CP	0.89	<0.001	0.9
	FOM	V _F , kn, NDF	0.71	<0.001	7.0
Groot	U	V _F , C, Tmax, ADF	0.92	<0.001	2.7
	kd	B, CP	0.67	<0.001	1.5
	FOM	V _F , B, NDF	0.85	<0.001	5.1

¹U, undegradable fraction; ²V_F, maximum gas production; kn, constant rate of gas production; Sn, specific rate of gas production; µm, maximum rate of gas production; Cg, fractional rate of gas production; A, constant factor of microbial efficiency; C, parameter determining the shape of the curve; Tmax, time to reach maximum rate of gas production; B, time at which 50% of V_F is reached.

There was a poor relationship between *in situ* parameters and gas production variables with R² varying from 0.36 to 0.65 for k_d estimation using the models of Groot and Logistic and from 0.42 to 0.50 for FOM estimation using the Logistic and Gompertz models. The wash out fraction (W) showed a constant relationship with starch and sugar contents (R² = 0.64). The transformation of k_d to its half-life value of degradation provided a slight improvement of its prediction for the model of Groot, with R² ranging from 0.67 to 0.79. There was an improvement of *in situ* parameters prediction when chemical composition of feedstuffs was used in the multiple regression analysis for all models (Table 1).

Conclusions The models showed differences in the predictive capability for some parameters. The chemical composition variables CP, NDF and ADF seem to be important to obtain good estimations of *in situ* degradation characteristics.

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Partial replacement of soyabean meal and rapeseed meal with a slow release urea source (Optigen® II) and its effect on microbial growth and metabolism *in vitro*

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Introduction Sources of non protein nitrogen (NPN) are attractive because of their low cost relative to vegetable proteins. Urea is the most commonly used NPN source and is rapidly hydrolysed to ammonia within the rumen. A very rapid release of ammonia may result in reduced utilisation by ruminal bacteria, increased N excretion, negative impacts on fertility and potentially ammonia toxicity (Richardson *et al.*, 2003). This has led to the development of slow-release urea based products including isobutylidene diurea, acetylurea, biuret and formaldehyde treated urea. Recently a blended fat and urea source (Optigen®) has been shown to reduce the rate of ammonia release in the rumen, but its effects on microbial metabolism are unclear. The objectives of the current study were to evaluate the effects of a slow release urea source (Optigen®) as a partial replacement for soyabean and rapeseed meal on microbial growth and metabolism *in vitro*.

Material and methods Four double layered glass *in vitro* fermentor vessels of 1.18 l volume as described by Sinclair *et al.* (2007) were used. Rumen fluid was removed from four ruminally cannulated sheep 4 h after the morning feed, diluted 1:0.75 with artificial saliva and added to each vessel. The vessels were maintained at 39°C with artificial saliva infused at the rate of 69 ml/h via a port in the head plate. Vessel contents were mixed using a motor controlled stirrer attached to the head plate for 5 min every 0.5 h at approximately 20 turns per min. Outflow was collected from a 20 mm internal diameter overflow port into a container maintained at 3 °C, and stored at -20°C. The vessels were fed 30 g DM/d of one of two dietary treatments in a 2 x 2 Latin square design, with each period lasting 14 d. The control diet contained (g/kg DM) 470 concentrate and 530 forage, of which (DM basis) 0.5 was grass silage and 0.5 maize silage. The concentrate consisted of (g/kg DM) 303 wheat, 303 sugar beet feed, 192 soyabean meal, 192 rapeseed meal and 10 of a standard minerals/vitamin mix. Optigen® (Alltech Inc. USA) was included at 5g/kg DM and replaced proportionally 0.27 of the soyabean meal and rapeseed meal, with the proportion of maize silage increasing accordingly. The feed was introduced via a 25 mm aperture in the head plate in three equal meals at 0800, 1600 and 0000 h. Days 1-8 of each period were used as an adaptation period with sampling occurring on days 9-14. Microbial protein synthesis was estimated by infusing ¹⁵N as a marker. The data were analysed by ANOVA as a 2 x 2 Latin square design, with all statistical analysis conducted using Genstat 10.1 (VSN Int. Ltd., Oxford, UK).

Results There was no effect ($P>0.05$) of treatment on mean fluid pH, volatile fatty acid (VFA) concentration or the ratio of acetate to propionate (Table 1). Fibre digestibility was 0.12 g/g higher and organic matter digestibility tended ($P=0.10$) to be higher in vessels receiving Optigen®. There was no effect ($P>0.05$) of treatment on microbial protein synthesis (gN/d) or the efficiency of microbial protein synthesis when expressed as gN/kg apparently degraded (OMAD) or truly degraded (OMTD), although numerically microbial flow was highest in vessels receiving Optigen®. Hourly ammonia concentrations were higher ($P<0.05$) in vessels receiving Optigen® (Fig 1.).

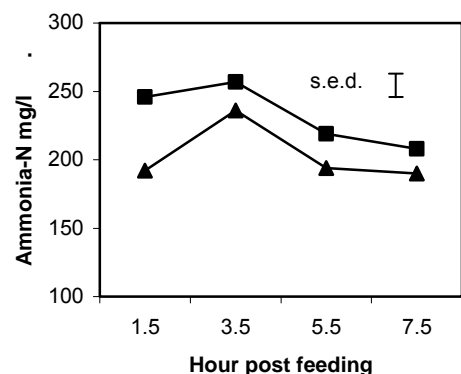


Figure 1 Effect of a Control diet (▲) or partial replacement of soyabean meal and rapeseed meal with Optigen® (■) on hourly NH₃-N concentrations

Table 1 Vessel fluid pH, volatile fatty acid (VFA) concentration, digestibility and efficiency of microbial protein synthesis when given a control diet (Control) or with the partial replacement of the soyabean meal and rapeseed meal with a slow release urea source (Optigen®).

	Control	Optigen®	s.e.d.	P-value
Mean fluid				
pH	6.21	6.16	0.128	0.717
VFA (mmol/l)	80.3	86.9	2.92	0.151
Acetate:propionate ratio	2.5	2.5	0.23	0.968
Digestibility (g/g)				
Organic matter	0.37	0.49	0.039	0.103
Fibre	0.45	0.57	0.028	0.046
Nitrogen (g/d)				
N intake	0.80	0.78		
Microbial-N output	0.35	0.39	0.118	0.748
Microbial protein synthesis ^a				
g N per kg OMAD	71.6	64.8	24.50	0.807
g N per kg OMTD	34.5	29.9	6.30	0.538

^aOMAD = organic matter apparently digested, OMTD = organic matter truly digested.

Conclusions There was no significant effect of Optigen® on microbial growth or VFA production but fibre digestion was increased. Optigen® therefore may represent a suitable alternative source of rumen degradable protein in ruminant diets.

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The effect of non-fibre carbohydrate on *in vitro* NDF disappearance of various ruminant feeds

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Introduction Type of supplemental carbohydrate provided in conjunction with forage has been suggested to be a factor that might impact the effect on NDF disappearance (Fondevila *et al.*, 2002). Of particular interest in this regard is the relative impact on fibre disappearance of supplementing with sugars versus starch (Arroquy *et al.*, 2005). The aim of the present study was to elucidate the impact of type of supplemental NFC (starch or sucrose) on *in vitro* NDF disappearance of lucerne, sugar beet pulp or wheat bran.

Materials and methods Experimental feed samples were lucerne, wheat bran, and sugar beet pulp. Samples were ground using a Willey-mill to pass 0.75mm screen, and dried at 80°C for 48h. Non-supplemented or non-fibrous carbohydrate (starch or sucrose) supplemented samples were incubated in a medium prepared as described by Arroquy *et al.* (2005). The supplementation was carried out as 70mg/g DM of feed samples of starch (St) or sucrose (Su) or a 1:1 composition of them (St+Su). 45ml of medium were distributed into 100ml bottles containing 1g of feed samples. Then, each bottle was inoculated with 5ml of strained rumen fluid and finely bubbled with CO₂. Three bottles for each treatment were incubated for 24, 48, and 96hrs at 39°C. Then, bottle contents were filtered through a 22µm filter, and NDF of the unfiltered medium was determined as described by Van Soest *et al.* (1991). Data of the disappearance rate of NDF were analysed using general linear model procedure of SAS (2003), with Duncan's test for the comparison of means (P< 0.05).

Results The effect of NFC supplementing on NDF disappearance of lucerne, sugar beet pulp, or wheat bran have been shown in Table 1. *In vitro* NDF disappearance of lucerne was significantly decreased by supplementing with Su or St (p< 0.05). While, it was not appear when sugar beet pulp was incubated. A significant effect of starch on *in vitro* NDF disappearance of wheat bran was recorded within 24hrs incubation. At 96hrs incubation, NDF disappearance of wheat bran was significantly decreased when St, Su or St+Su was added to the medium (p< 0.05).

Table 1 *In vitro* NDF disappearance (g/ Kg) of lucerne, sugar beet pulp or wheat bran supplemented by starch, sucrose or starch +sucrose.

Incubation time (h)	Feed	Non-supplemented	NFC sources			SEM ¹	P ²
			Sucrose*	Starch**	Sucrose +starch***		
24	Lucerne	175.4 ^a	81.9 ^b	105.3 ^b	61.3 ^b	6.24	< 0.01
48	Lucerne	187.1 ^a	101.7 ^b	119.9 ^b	116.9 ^b	4.41	< 0.01
96	Lucerne	345.0 ^a	204.7 ^{bc}	274.8 ^{ab}	181.3 ^c	6.94	< 0.01
24	Wheat bran	129.7 ^a	122.2 ^a	182.2 ^b	197.2 ^b	5.87	< 0.05
48	Wheat bran	317.3	377.3	332.3	347.3	5.87	0.12
96	Wheat bran	474.8 ^a	369.8 ^b	287.3 ^b	317.3 ^b	8.12	< 0.05
24	Sugar beet pulp	286.2	214.8	200.6	279.1	8.29	0.20
48	Sugar beet pulp	371.9	314.8	336.2	336.2	6.85	0.37
96	Sugar beet pulp	357.6	393.3	357.6	329.1	7.70	0.47

^{a,b,c} within a row, means without the common superscript letter differ.

Sucrose was added as 70 mg/ g DM; ** Starch was added as 70 mg/ g DM; *** 35 mg/ gDM of sucrose and 35 mg/ g DM of starch was added ¹ Standard error of mean ² P-value

Conclusions Results of the present study indicated that *in vitro* NDF disappearance of lucerne and wheat bran, as a fibrous feedstuffs, are influenced by the NFC. *In vivo* work reported by Heldt *et al.* (1999) indicated that supplementation with starch had a more negative effect on forage fibre disappearance than did simple sugars. Based on the data obtained in the present study, it was concluded that, generally, NFC supplemented fibrous feedstuffs had lower *in vitro* NDF disappearance when samples were incubated for 96hrs. In addition, the effect of NFC on the amount of NDF disappearance resulted from different type of feedstuffs is not similar and it might be related to the nature of the incubated feeds.

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The study of fermentation characteristics of some by-products using gas production technique

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Introduction The association between rumen fermentation and gas production has long been known. The gas measuring technique has been widely used for evaluation of nutritive value of feeds. Gas measurement provides a useful data on digestion kinetics of both soluble and insoluble fractions of feedstuffs (Getachew *et al.*, 1998). In the gas method, kinetics of fermentation can be studied on a single sample and therefore a relatively small amount of ample is required or a larger number of samples can be evaluated at time. When a feedstuff is incubated with buffered rumen fluid *in vitro*, the carbohydrates are fermented to short chain fatty acids (SCFA), gases (mainly CO₂ and CH₄) and microbial cells. Gas production is basically the result of fermentation of carbohydrates to acetate, propionate and butyrate (Beuvink and Spoelstra, 1992; Blümmel and Ørskov, 1993). The purpose of this study was to valuate of nutritive value of grape marc (GM), tomato pomace (TP), grape pomace (GP) and raisin waste (RW) using gas production technique.

Materials and methods Samples of rumen fluid were collected from two fistulated sheep fed twice daily a diet containing forage (400 g/kg) plus concentrate (600 g/kg) after morning feeding, strained through four layers of cheesecloth. Gas production was measured by Fedorak and Hrudý (1983) method. Approximately 300 mg of dried and ground (2mm) feeds samples were weighted and placed into serum bottles. Buffered rumen fluid whit McDougal buffer (20ml) was pipetted into each serum bottle (McDougal 1948). The gas production was recorded after 2, 4, 6, 8, 12, 16, 24, 36, and 48 96 h of incubation. Total gas values were corrected for the blank incubation, and reported gas values are expressed in ml per 200 mg of DM. The data at the different times was analyzed using completely randomized design by the GLM procedure of SAS Institute Inc (2002). The gas production profiles in triplicate fitted with equation of $Y = A(1 - e^{-ct})$ where Y is the volume of gas production (ml/g DM) at time t , A is gas production from soluble and insoluble fraction, c is the gas production rate and t is the incubation time (h). Samples of feeds were collected from food factories in the country of Tabriz.

Results The results are showed in Table 1. Results indicated that the gas production volume of test feeds in incubation time (48 h) was different significantly ($p < 0.05$). The gas production volume of grape pomace was higher than other samples, and raisin waste had the lowest gas production volume within the feedstuffs. The rate of gas production (c) of grape pomace was the higher than other feeds. Also grape pomace has the highest potential of gas production (A) within the samples ($p < 0.05$).

Table 1 Gas production characteristics *

	Feeds				SEM
	GM	GP	RW		
A ¹ (mL/g DM)	221 ^b	259.3 ^a	213.9 ^c	0.524	
c ² (/h)	0.105 ^b	0.120 ^a	0.088	0.002	
TGP ³	243 ^b	270 ^a	208 ^c	6.218	
ED ⁴	209.24	237	175.59		

Table 2 Chemical compositions of feeds (%)

feeds	items		
	DM	CP	ADF
GM	88.54	6.5	25.6
GP	93.24	6.6	18.4
RW	92.78	5.8	23.4

* The means within a row without common letter differ ($p < 0.05$).

- 1- The potential of gas production
- 2- The rate of gas production (/h)
- 3- Total gas production at time 48 h
- 4- Effective degradability (mL/g DM)

Conclusion The *in vitro* gas production technique can be used to determine the nutritive value of the feedstuffs and to identify differences among their potential digestibility. The results showed that the differences between chemical compositions of feedstuffs caused to change fermentation parameters determined by *in vitro* gas production technique. These results indicated that difference characteristics of degradability of feedstuffs and must be that considered in diet formulation in ruminant.

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Biological technique of upgrading the feeding quality of wheat straw

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Introduction Wheat is vastly grown worldwide in order to feed a growing population and for use as ruminant feed. In developed countries where the value of wheat has increased, farmers are growing varieties which have a shorter, thicker stem to structurally support the high yielding characteristics. Straw produced from such varieties is highly lignified and is harder to digest by ruminants. Several techniques have been employed in attempt to improve the nutritional value and digestibility of wheat straw for ruminant feeding, but with no success; e.g. due to the impalpability of chemically-treated straw, and the expense (and often high labour input) involved in highly technical physical techniques. It has already been proven that the *Pleurotus* species of fungi can significantly degrade the lignin content of many lignified substances (Cohen *et al.*, 2002). *Pleurotus* species can selectively degrade the lignin content of wheat straw, resulting in the cellulose and hemicellulose becoming more accessible, thus allowing the rumen micro-organisms to digest the substance successfully. It is also suggested that the addition of Manganese (Mn) can enhance the degradation of lignin (Hadar & Kerem, 1995). The aim of this study was to investigate the effect of three concentration levels of Manganese in addition to two strains of *Pleurotus* fungi (*ostreatus* 027 and *eryngii* DSM8264) in order to determine the best combination to achieve increased digestibility and lignin degradation of wheat straw.

Materials and methods The wheat straw employed was bought in the form of three small bales. All of the straw was inserted into a fully sterilized big bale chopper to achieve 7 cm straw lengths. Degradation takes place via the lumen of the straw rather than the outer parts thus chopping the straw means the lumen becomes more accessible for the fungi to colonise; hence improve colonisation. 300 g of the straw was placed into each of the 24 autoclavable polythene bags along with 132 ml of distilled water to achieve a moisture content of 60%. Bags were then autoclaved (121°C, 15 psi, 20 min) then inoculated with the appropriate strain of *Pleurotus* fungi. The fungi were cultivated and maintained on 4% malt agar plates in the dark for 14 days, before being pre-cultured on a mixture of barley and wheat grains to be used as an introductory medium in order to inoculate the bags. Six bags were kept as controls (no Mn introduced); another 9 bags were inoculated with *P. ostreatus*, which were then sub-divided into 3 × 3 bags receiving 31, 125, or 500 µg of Mn. Exactly the same method was used to inoculate the other nine bags with *P. eryngii*. After inoculation, bags were heat-sealed and incubated for 8 weeks (27°C, relative humidity 85%). During incubation, bags were carefully monitored to ensure that fungal growth took place. Cotton windows inserted into the polythene bags provided adequate ventilation. The experiment did not run long enough for the fungi to develop fruiting bodies. All treated straw samples were milled to reach a 1mm fine flour consistency prior to chemical analysis. In-vitro dry matter digestibility was determined by the Modified Faeces Liquor Method (Omed *et al.*, 1989). Lignin and crude protein content were determined by modified techniques (Halliday, 1985). The statistical models used were ANOVA and Paired Sample T-Tests (SPSS v.12.5).

Results Table shows Mean ± SE values for crude protein and in-vitro dry matter digestibility (IVDMD) for each fungi and Mn level (n = 6) as well as the ANOVA for the digestibility values per Mn level and fungi species (n = 3).

Fungi (strain)	Mn (µg)	level	Crude protein (%)	IVDMD	ANOVA (p value) for digestibility per Mn level per spp.
<i>P. ostreatus</i>	0		2.39 ± 0.13	36.9 ± 0.2	
<i>P. ostreatus</i>	31		4.09 ± 0.11	37.6 ± 0.5	.114
<i>P. ostreatus</i>	125		4.11 ± 0.06	39.6 ± 0.6	
<i>P. ostreatus</i>	500		2.43 ± 0.04	41.5 ± 0.4	
<i>P. eryngii</i>	0		2.43 ± 0.23	40.2 ± 0.3	
<i>P. eryngii</i>	31		3.01 ± 0.47	38.8 ± 0.5	.176
<i>P. eryngii</i>	125		2.56 ± 0.62	31.8 ± 0.5	
<i>P. eryngii</i>	500		2.98 ± 0.39	31.6 ± 0.5	
Control (untreated straw)	0		2.07 ± 0.46	44.3 ± 9.1	

Most of the fungi and different levels of Mn either reduced the in-vitro digestibility or made no difference; but the *P. ostreatus* increased in the in-vitro digestibility of straw with the addition of Mn compared to the *P. ostreatus* with no Mn control. Crude protein levels were increased with each fungi strain and Mn level. Crude protein values of the *P. ostreatus* proved significantly higher ($p = 0.027$) than non-treated straw. Straw treated with *P. eryngii* showed a lower remaining level of lignin. The One-Way ANOVA showed significant variability within each level of Mn used for each fungi species.

Conclusion Growth of *Pleurotus* species proved effective on wheat straw. *P. ostreatus* can be considered as a candidate for improving the in-vitro digestibility of wheat straw as results showed that with the addition of Mn, *P. ostreatus* had a higher crude protein and in-vitro digestibility value than other fungi strains. However, lignin and cellulose analysis suggest that *P. eryngii* was more of a selective type of fungi. In addition to improving the digestibility of the straw, there is scope to create an extra source of income for farmers from the sale of an edible crop of cultivated fungi. However, as all but one result were not considered significant, further work needs to be performed on these fungi strains and the importance of Mn addition needs to be elucidated.

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Evaluation of some by-products using *in situ* technique

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Introduction The dynamic interactions within the rumen are difficult to simulate. *In situ* technique allow us to study digestion within the rumen and reduce the need for ruminal simulation. The nylon bag technique has been used for many years to provide estimates of both the rate and extent of disappearance of feed constituents (Mehrez and Ørskov, 1977). This technique provides a useful means to estimate rates of disappearance and potential degradability of feedstuffs and feed constituents. The technique has also provided relatively good predictions of forage intake and digestibility (Ørskov, *et al.*, 2000) and has greatly improved the understanding of nitrogen (N) supply to ruminants and their microbes. By-product feeds are produced by the wet milling, brewing, and other food or feed processing industries and have been recognized as potential feed sources for decades. Feeding by-products to dairy cows and other livestock will probably continue to increase in the future. By-product feeds fed to dairy cows and other livestock prevents a waste disposal problem for industry and reduces the amount of concentrate that must be included in the diet to ensure maximum animal performance. The object of this study was to evaluate of nutritive value of grape marc (GM), noodle waste (NW), tomato pomace (TP), apple pomace (AP), grape pomace (GP) and raisin waste (RW) using nylon bag technique.

Materials and methods *In situ* degradation characteristics were determined (Ørskov and McDonald, 1970) in triplicate in 2 ruminal fistulated sheep (38±1.5). The sheep were fed twice daily a diet containing 600 g/kg concentrate and 400 g/kg forage. Nylon bags which were approximately (6×12 cm) containing 5 g (2mm screen) were incubated in duplicate in the rumen of fistulated sheep for 0,2,4,6,8,12,16,24,36 and 48 h. There were 4 replication per treatment. The rate and extent of DM degradation were estimated according to the equation: $P = a + b(1 - e^{-ct})$. Effective degradability (ED) was calculated according to equation: $ED = a + (b \times c) / (c + k)$, assuming an outflow rate (k) of 0.02 h⁻¹. The data was analyzed using the ANOVA procedure of SAS (SAS Institute, 2002).

Result The results are shown in Table 1. There was a significant differences between treatment in incubation times ($p < 0.05$). DM disappearance of grape pomace (GP) at 0, 2 and 4 h was higher than other feedstuffs. But after 6 h incubation noodle waste has the highest DM disappearance value within the feedstuffs until 48 h incubation. Tomato pomace has the lowest dry matter disappearance value within the feedstuffs at all the incubation times. DM disappearance value between raisin waste and grape marc was not significantly different but grape pomace has a higher DM disappearance value at 48 h. Grape pomace and tomato pomace have the highest and lowest *a* value (soluble fraction) within the feedstuffs, 68.92% and 13.55% respectively. Insoluble fraction (*b*) and disappearance rate for noodle waste were more than the other feedstuffs. Noodle waste and tomato pomace have the highest and lowest effective disappearance (ED) within the feedstuffs.

Table 1 *In situ* disappearance(%) of DM*

Feeds	Time of incubation (h)										a	b	c (%/h)	ED
	0	2	4	6	8	12	16	24	36	48				
GM	51.2 ^b	51.4 ^b	54.2 ^b	55.9 ^c	56.8 ^c	57.9 ^c	60.5 ^c	60.7 ^d	63.1 ^d	65.6 ^d	52.62	22.02	.0212	63.9
GP	68.9 ^a	69.2 ^a	70.4 ^a	70.8 ^b	71 ^b	71.1 ^b	71.3 ^b	75.9 ^b	76.2 ^b	78.4 ^b	68.92	23.64	.0107	77.2
RW	50.3 ^b	50.7 ^b	54 ^b	55.2 ^c	56.3 ^c	57.2 ^c	56.2 ^d	58.1 ^d	60.8 ^d	63.6 ^d	51.13	13.37	.0405	60.1
NW	20.7 ^d	32.6 ^c	54.4 ^b	80.4 ^a	80.8 ^a	80.7 ^a	81.8 ^a	83.9 ^a	97.6 ^a	97.8 ^a	17.13	75.03	.1942	85.2
AP	46.9 ^c	50.6 ^b	51.8 ^b	51.9 ^d	53 ^c	56.5 ^c	57.8 ^d	69.2 ^c	71.1 ^c	74.2 ^c	46.96	39.85	.0255	69.3
TP	15.1 ^e	15.6 ^d	17.6 ^c	18.3 ^c	19.9 ^d	24.5 ^d	30.6 ^e	32.9 ^e	42.3 ^e	45.3 ^e	13.55	51.76	.0208	39.9
SEM	0.379	1.794	0.848	1.022	1.983	1.563	0.888	1.063	1.537	1.282				

*The means within a column without common letter differ ($p < 0.05$).

Conclusion In the present study, feeds composition significantly affected the degradation parameters, when ruminal DM degradation of the various feed samples were considered. Results of the present experiment indicated that the rapidly degradable fraction (a) of DM of GP was higher than other feeds. These results indicated that by-products of food factories can be used as replacement feedstuffs in diet for ruminants, but it needs to more research.

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The potential of low temperature steam treatment for improving the nutritional value of sugarcane pith

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Introduction The key to maximising the nutritional value of lignocellulosic materials is in disrupting the plant cell walls as to allow complete access to nutrients and not creating extra anti-nutritional factors. The disruption conditions of choice will always be a compromise between severe processes that achieve high levels of access, but simultaneously form anti-nutritional factors, and milder but less disruptive. Sugarcane pith is highly lignified by products of the sugar and paper industries. For treatments by steam and pressure alone, harsh conditions are needed ($t > 180^\circ\text{C}$). Under these conditions acetyl groups are released from the hemicellulose matrix and suitable levels of cell wall disruption are achieved, also result in formation of furfural by secondary dehydration reactions of hemicellulosic pentoses and soluble phenolic compounds. Both of them inhibit the activity of rumen microbes and cell-free enzymes. Using lower temperatures with an acid can achieve comparable cell wall disruption to steam treatment at high temperatures (Grohmann *et al.*, 1985), and results lower amounts of toxic compounds. The objective of this experiment was to evaluate the effects of low temperature steam treatments with sulphuric acid (H_2SO_4) concentrations and reaction times on utilisation of sugarcane pith by rumen microbes.

Materials and methods Sulphuric acid solution was added to ground sugarcane pith (100 g; of about 92% DM) to obtain samples of approximately 30% dry mater (DM) content with 0, 0.6, 1.2 and 1.8% H_2SO_4 on a DM basis. Then, pith samples were autoclaved at 121°C and 134°C for 40, 80 and 120 min. The pressures at 121°C and 134°C were 1.1 and 2.2 bar, respectively. The samples were oven-dried overnight at 55°C . Gas production (GP) was analyzed in triplicates by the Menke and Steingass (1988) technique using 100 ml glass syringes. Syringes were incubated in 39°C and GP measured at 3, 6, 12, 24, 48, 72 and 96 h. The cumulative *in vitro* gas production data was fitted to the exponential equation $Y = b(1 - e^{-ct})$.

Results *In vitro* gas production of steam treated sugarcane pith is shown in Table 1 and 2. The treatment conditions tested in this study, temperature, acid concentration and reaction time affected potential of gas production. Increased in amount of acid, temperature and reaction time resulted in a significant increase of GP ($P < 0.05$). The most and the least amount of gas production (111 ± 3 vs. 72 ± 0.9 per 300 mg DM) was for highest and lowest amount of temperature (134 and 121°C), acid (1.8 and 0 per DM) and reaction time (120 and 40min), respectively ($P < 0.05$).

Table 1 Cumulative *in vitro* GP (ml per 300 mg DM) of steamed sugarcane pith (mean \pm s.e.)

Treatments condition		GP	Acid, % dry mater			
Temp, $^\circ\text{C}$	time, min.		0	0.6	1.2	1.8
134	120	<i>b</i>	78.4 \pm 2	80.4 \pm 1.6	107 \pm 2.6	111 \pm 3
		<i>c</i>	0.06 \pm 0.001	0.03 \pm 0.001	0.04 \pm 0.002	0.04 \pm 0.008
134	80	<i>b</i>	74 \pm 0.1	84.6 \pm 3	92 \pm 1.4	107 \pm 5
		<i>c</i>	0.06 \pm 0.002	0.03 \pm 0.002	0.05 \pm 0.002	0.03 \pm 0.004
134	40	<i>b</i>	72.8 \pm 1	72.6 \pm 0.7	79.2 \pm 0.7	95.3 \pm 0.9
		<i>c</i>	0.07 \pm 0.002	0.02 \pm 0.004	0.05 \pm 0.003	0.06 \pm 0.002
121	120	<i>b</i>	77.5 \pm 3	77.9 \pm 3	82 \pm 1.5	99.2 \pm 4
		<i>c</i>	0.03 \pm 0.0	0.03 \pm 0.005	0.04 \pm 0.004	0.02 \pm 0.002
121	80	<i>b</i>	72.4 \pm 0.5	73.4 \pm 0.6	77.9 \pm 1.5	85.4 \pm 1.1
		<i>c</i>	0.05 \pm 0.002	0.04 \pm 0.002	0.04 \pm 0.004	0.04 \pm 0.003
121	40	<i>b</i>	72 \pm 0.9	74.8 \pm 2	80.4 \pm 0.5	86.5 \pm 1
		<i>c</i>	0.02 \pm 0.003	0.06 \pm 0.006	0.03 \pm 0.002	0.1 \pm 0.005

b = GP from fermentable fraction *c* = rate constant of GP

Table 2 The main effects of acid, temperature and reaction time on cumulative GP (ml per 300 mg) of sugarcane pith

GP	Acid, g/kg DM				s.e.m	temperature, $^\circ\text{C}$		s.e.m	Time, minute			s.e.m
	0.0	6	12	18		121	134		40	80	120	
	74.5 ^c	77.3 ^c	86.5 ^b	97.4 ^a		0.23	80 ^b		87.9 ^a	0.16	79.3 ^b	

Conclusions The results of the present study demonstrate that treatments of acidified sugarcane pith with low temperature particular in hard condition (134°C for 120 min. and 1.8% acid per DM) improved GP and therefore its utilisation for rumen micro organisms. Amount of GP in this condition is comparable with GP of the sugarcane pith treated in high-pressure (210°C , 3 min. 19 bar) (122.5 vs. 111) (observation of authors, unpublished data).

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Effects of alfalfa particle size and soybean oil on digestibility, chewing activity, milk yield and composition of early lactating Iranian Holstein cows

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Introduction The supplemental fats can reduce milk fat and protein percentage and increase milk yield, because the addition of fat can interfere with ruminal fermentation result in decreased fibre digestion. By the way, forage particle size, however, is closely related to ruminal lipid metabolism in several ways including increase in the size and consistency of ruminal mat, increase ruminal mean retention time of the particulate and lipid and its availability for microbial attack and to reduce the rate of lipid hydrolysis and biohydrogenation, which could potentially lower and release of antimicrobial fatty acids. Jenkins *et al.* (1998) reported a tendency for increase production of FCM when tallow was added to diets with short-cut hay, which suggests at least a limited role of hay particle length. In addition several researchers reported that reduction of forage particle size cause increased DMI, milk yield and milk protein but decreased chewing activity, rumination, Intake time, rumen pH, milk fat percentage and fibre digestibility (Teimouri Yansari *et al.*, 2004). Therefore, this study was conducted to evaluate the effects of alfalfa hay particle size and soybean oil on nutrient digestibility, chewing activity, milk yield and composition of early lactating Iranian Holstein cows.

Material and methods Four lactating Holstein dairy cows (DIM= 60d) were allotted to 4×4 repeated Latin square design at four 21d periods (adaptation, 14 d, and sample collection, 7 d) to evaluate the effects of two levels of particle size (fine and coarse) and two levels of soybean oil (0 and 4 %) on the dry matter intake, nutrients digestibility and chewing activity and performance of Holstein Dairy cows. Samples of silage, rations, and faeces were dried at 55 °C for 48 h in a forced-air oven for DM, ground through a Wiley mill (1-mm screen pore size), analyzed for DM, CP, EE, ash, NDF. Non fibrous carbohydrate was calculated by 100- (%CP+ %NDF+ %Ash+ %EE). Eating and ruminating activities were monitored visually for cows in the treatments over a 24 h period for one day of each experimental period.

Results and discussion The results of experiment are presented in Table 1 and 2. The diets had similar chemical composition and energy. Reduction of particle size significantly increased DMI, but oil supplementation did not affect on DMI. In addition, reduction of particle size and oil supplementation decreased digestibility of DM, NDF, EE, eating time, rumination time, and total chewing activity. Also, reduction of particle size significantly increased milk yields, but oil supplementation significantly increased it that may be because of increased and decreased DMI, respectively. In addition, reduction of particle size significantly decreased milk fat and increased milk protein, in contrary the oil supplementation significantly decreased and increased milk fat and protein, respectively. It seems that coarse particle can form a consistent ruminal mat, stimulate chewing activity, improve ruminal environment and cell wall degradation, and finally increase milk fat, therefore coarse forage is a good source of physical effective fibre.

Table 1 Ration ingredients and nutrient composition.

	Diet with 0% oil	Diet with 4% oil
Ingredient (%)		
Corn silage	20.0	20.0
Alfalfa hay	20.0	20.0
Beet pulp	7.5	7.8
Barley grain	30.0	26.5
Wheat bran	10.0	10.0
Cottonseed meal	11.0	10.0
Urea	0.2	0.2
DCP	0.6	0.6
Mineral premix	0.4	0.4
Salt	0.3	0.5
Soybean oil	0.0	4.5
Chemical composition (%)		
NDF	33.11	31.51
ADF	19.11	18.69
CP	16.21	15.71
EE	3.00	6.51
NE _L , Mcal/kg	1.65	1.67

Table 2 Intake, digestibility, chewing activity and performance.

	Treatments			
	4 Coarse	4 Fine	0 Coarse	0 Fine
Oil Level				
Particle size	Coarse	Fine	Coarse	Fine
Dry matter intake (kg/d)	21.2 ^b	23.28 ^a	21.32 ^b	22.65 ^a
Digestibility of DM (%)	67.84 ^a	63.35 ^b	69.94 ^a	64.32 ^b
Digestibility of NDF (%)	55.93 ^b	48.83 ^c	61.2 ^a	56.01 ^b
Digestibility of CP (%)	74.44 ^a	76.09 ^a	68.11 ^b	70.16 ^b
Digestibility of EE (%)	62.08 ^b	54.18 ^c	66.02 ^a	64.12 ^{ab}
Eating (min/d)	260 ^{ab}	210 ^b	278.5 ^a	225 ^{ab}
Rumination (min/d)	438.7 ^{ab}	370 ^c	472.5 ^a	396 ^{bc}
Total chewing activity(min/d)	698.7 ^{ab}	580 ^c	751 ^a	621 ^{bc}
Milk yield (kg/d)	28.71 ^{ab}	30.01 ^a	26.81 ^c	27.87 ^{bc}
Milk fat (%)	3.42 ^b	3.28 ^d	3.56 ^a	3.36 ^c
Milk protein (%)	2.79 ^b	2.81 ^b	3.05 ^a	2.97 ^a

Means within a row with different superscripts differ (P<0.05).

Conclusion Base the results of current experiment, it can be concluded that coarse particles are a good source of physical effective fibre. However, oil supplementation in ration can not be considered as a source of effective fibre because oil supplementation reduced milk fat.

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Effect of fumaric acid supplementation on nutrient digestibility in growing Kadon pigs

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Introduction The aim of the present study was to determine whether the addition of fumaric acid (FA) to the diet would influence nutrient digestibility in growing Kadon pigs. In pigs there is substantial fermentation in the intestinal tract. To monitor whether the feeding of fumaric acid had an effect on microbial activity, were analyzed the concentrations of volatile fatty acids (VFA) in intestinal contents.

Materials and methods The feeding trial had a 3x3 Latin square design. The three experimental treatments were diets either without (control) or with 3.0 or 6.0 % FA. Six growing Kadon pigs, aged three months, were used. The control diet was a complete, commercial starter diet containing about 18 % protein. To produce the experimental diets, the commercial starter diet was mixed with either 3 or 6 % of FA. Feed refusals, if any, were recorded and weighed. At the beginning of each period, body weights (BW) of the pigs were determined. Feed and feces samples were analyzed for macronutrients (AOAC, 1990).

The pigs were killed on the last day of the experiment. The gastro-intestinal tract (GIT) was divided into stomach, small intestine and large intestine. The digesta of each compartment was properly mixed and samples were collected for pH and analysis VFA. pH of intestinal contents was determined using a pH meter (Model HI99163, Hanna Instruments, Portugal). VFA concentrations in the intestinal contents were analyzed by HPLC (Shimadzu Class-VP), using a Aminex® column (HPX-87H, 300m x 7.8 mm), UV detector and N₂ as carrier gas at 190 °C. Iso-caproic acid was used as an internal standard. The data were subjected to analysis of variance for a 3x3 Latin square design, using the General Linear Model of SAS Software (1985). Duncan's multiple comparisons test was used to determine differences between treatment means. The level of statistical significance was preset at $P < 0.05$.

Results and Discussion BW gains of the pigs ranged from 1.8 to 4.1 kg over the entire experimental period. There was no diet effect on feed intake, average daily gain (ADG), feed conversion ratio (FCR) and final BW (Table 1). The ingestion of fumaric acid had no significant effect on the apparent fecal digestibility of macronutrients (Table 2).

Table 1 Effect of fumaric acid (FA) supplementation on growth performance in growing Kadon pigs

Item	Control	3%FA	6%FA	SEM
Initial BW, kg	21.1	20.4	20.8	0.867
Final BW, kg	24.1	23.8	23.7	0.869
ADG, g/d	235.0	285.0	245.8	12.826
DMI, g/d	982.3	1053.7	1006.9	60.815
FCR	4.1	3.7	4.3	0.270

propionate, butyrate and total VFAs in the small intestine. The butyrate concentration in the large intestine section was decreased by FA supplementation in a dose-dependent fashion (Table 3).

Conclusion In this study the supplementation of the diet with fumaric acid did not affect the digestibility of macronutrients in growing Kadon pigs. Dietary fumaric acid did influence bacterial activity in the hindgut as based on the changes in VFA concentrations in large intestinal contents. The impact of this observation is not known.

Table 2 Effects of fumaric acid (FA) supplementation on macronutrient digestibility in growing Kadon pigs

Item	Treatment			SEM
	Control	3% FA	6% FA	
Digestibility, % of intake				
DM	89.1	89.3	90.7	0.733
OM	88.1	89.2	91.2	0.791
CP	85.3	85.7	86.8	1.110
EE	83.8	86.0	89.1	1.229
CF	64.3	62.8	70.2	2.285
NFE	90.9	92.1	93.6	0.632
Ash	61.5	67.0	66.6	2.358
Gross energy	84.8	86.3	88.5	0.995

Table 3 Effect of FA on VFA concentrations in various sections of the GIT of growing Kadon pigs

Item	Treatment			SEM
	Control	3% FA	6% FA	
Stomach				
pH	4.17	3.95	4.16	0.300
Acetate, mmol/l	0.54 ^b	0.26 ^a	4.47 ^c	0.038
Propionate, mmol/l	4.08	4.07	4.59	0.070
Butyrate, mmol/l	2.69 ^a	3.16 ^{ab}	3.31 ^b	0.070
Total, mmol/l	6.67 ^a	7.25 ^a	12.36 ^b	0.095
Small intestine				
pH	6.82	6.40	6.84	0.093
Acetate, mmol/l	39.09	52.90	49.60	3.054
Propionate, mmol/l	14.16	15.58	12.90	0.449
Butyrate, mmol/l	4.21	2.95	2.59	0.199
Total, mmol/l	57.46	71.43	65.09	3.418
Large intestine				
pH	6.19	6.48	6.10	0.171
Acetate, mmol/l	36.61 ^{ab}	19.12 ^a	48.91 ^b	3.509
Propionate, mmol/l	4.00	4.30	4.02	0.090
Butyrate, mmol/l	62.14 ^b	55.87 ^b	38.58 ^a	1.692
Total, mmol/l	102.75	79.28	91.51	5.119

^{a,b} Means in the same rows with different superscripts differ ($P < 0.05$)

Increasing intakes of fumaric acid tended to be associated with increased digestibilities of NFE and GE. The concentration of acetate in the stomach was significantly different between treatments. The pigs fed the diet with 6.0% fumaric acid had significantly higher acetate, butyrate and total VFAs in their stomach contents than did pigs fed either the control diet or the diet with 3.0% fumaric acid. Between treatments there was no significant difference in pH and the levels of acetate,

Effects of maize cultivar and dry matter content at harvest on rumen fermentation properties

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Introduction Forage maize is mainly composed of starch in the ears and cell walls in the stover. Upon feeding maize to cattle starch can be fermented in the rumen, but after bypassing the rumen it can also be digested in the intestines. There is not much knowledge on the influence of maturation of the maize plants on the amount of rumen fermentable starch and on the kinetics of the rumen degradable part. There is also not much insight in the variation in rumen fermentation properties by different genotypes (cultivars). In many feed evaluation systems the total cell wall degradability of maize is valued and not its rate of degradation in the rumen. To get insight in the changes in cell wall and starch quality 4 different maize cultivars were harvested during the growing season and analysed for chemical composition and rumen fermentation kinetics, determined with the gas production technique.

Materials and methods Four maize cultivars were harvested at dry matter (dm) contents of 250 (dm250), 320 (dm320) and 390 g/kg (dm390). Cultivar 1 (cv1) was an early dry down cultivar, cv2 early stay green, cv3 late dry down and cv4 was a late stay green cultivar. Plants were separated in ears and stover, freeze dried and ground to pass a 1 mm screen. Samples of 0.5 g dm were incubated in the gas production technique as described by Cone *et al* (1996). Non-lactating donor animals received 9 kg DM grass hay and 1 kg DM concentrate daily. Gas production profiles were run in duplicate and fitted to a three-phasic model as described by Groot *et al* (1996). Presented are values for gas production after 20 h (GP20) and for A1 and A2, gas production caused by fermentation of the soluble and insoluble fraction respectively and B2 the time (h) needed to reach half of A2. Starch degradation after 10 h was calculated using gas production and starch content as described by Chai *et al* (2004). Statistical differences were recognised using analysis of variance.

Results Table 1, showing the main effects of the research, displays starch content, starch degradation after 10 h incubation and gas production parameters for the ear and stover samples. Dry matter content at harvest showed significant effects on all parameters. Cultivar was not significant for starch content and B2 in ears. Effects for dry matter x cultivar were only observed in ears for GP20, A1, A2 and degraded starch.

Conclusions Early harvest results in low starch contents and decreased fermentation of the non-soluble fraction (A2). Upon maturation (higher dm content) rate of degradation of both starch and cell wall decreased (higher B2). This lead to a decrease in percentage starch degradation after 10 h incubation, which indicates a higher percentage of rumen escape starch. Prolonged maturation results in decreased cell wall degradability (low A2) and decreased rate of degradation (high B2). Expanded studies should point the optimum harvest date (dm content) to obtain maize with high yield, high starch content and high cell wall degradability and optimal starch degradation in the rumen with desired amounts of rumen escape starch and maximum digestibility in the intestine.

Table 1 Content of starch (g/kg DM), gas production (ml/g OM) after 20 h (GP20), caused by fermentation of the soluble fraction (A1) and the insoluble fraction (A2), time (h) needed to reach half the maximum of A2 (B2) and the starch degraded (g/kg) after 10 h incubation.

	Starch	GP20	A1	A2	B2	Starch degr.
	g/kg DM	ml/g OM	ml/g OM	ml/g OM	h	g/kg
Maize ears						
dm250	426	299	45.6	253	7.25	771
dm320	630	310	28.2	282	7.54	665
dm390	648	309	23.9	285	7.77	619
lsd	29	5.2	1.9	4.2	0.23	13
cv1	576	304	32.9	271	7.40	682
cv2	563	312	32.1	280	7.61	694
cv3	546	305	37.0	268	7.44	701
cv4	586	303	28.3	275	7.64	650
lsd	33	6.0	2.2	4.8	0.27	15
Effect dm	***	***	***	***	**	***
Effect cv	ns	*	***	***	ns	***
Effect dm x cv		*	**	*	ns	**
Maize stover						
dm250		210	52.1	158	8.47	
dm320		193	38.3	155	9.05	
dm390		191	43.3	147	9.40	
lsd		6.3	2.9	4.8	0.15	
cv1		190	40.1	150	9.34	
cv2		193	39.2	154	8.96	
cv3		211	52.8	158	8.84	
cv4		197	46.1	151	8.76	
lsd		7.2	3.3	5.6	0.17	
effect dm		***	***	**	***	
effect cv		***	***	*	***	
effect dm x cv		ns	ns	ns	ns	

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The effects of steaming and soaking treatments on respirable particles and nutrient levels of hay

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Introduction Hay is a very common forage fed to horses in the UK with Hollands (2001) reporting it as the most common. The affiliation between hay and equine respiratory disease is well documented and McGorum (2001) states that 90% of hay made in the UK is of inadequate quality for consumption by horses. The process of soaking hay prior to feeding is well recognised to reduce the number of spores within the hay (Blackman and Moore-Colyer, 1998; McGorum, 2001). As a consequence soaking hay is common practice in many stable yards. This has many disadvantages including disposal of the post-soak liquor and the reduction in nutrient value of the hay (Blackman and Moore-Colyer, 1998). Steaming hay has been suggested as a potential alternative to soaking. There are currently few studies directly comparing the efficacy of soaking and steaming. This study aims to compare the impact of both soaking and steaming treatments on the levels of respirable particles and nutrients of hay.

Materials and Methods Ten 100g samples were weighed out from each of four bales of hay intended for equine consumption. From each bale two samples were kept as controls and the remainder were soaked or steamed for 30, 60, 80 or 90 minutes. Soaked samples were submerged in 24 litres of cold water for the designated time period and then hung to drain for 15 minutes prior to particle collection. Steamed samples were steamed using a domestic steamer (Cookworks SO119), above 1.5 litres of water, at 100°C for the designated time period and then left to cool (but not dry out) for 15 minutes, prior to particle collection. All hay samples were then placed in a container which was mechanically shaken for 6 minutes under a suction and filter unit with a sub-micro filter and 8 µm fixed pore size filter paper (adapted from Moore-Colyer, 1996). A 1 cm² area of filter paper was counted for particles (after 48 hours of Triacetin to clear it). The number of respirable particles (RP) per gramme of hay was then calculated for each sample. Dry matter was determined and Water Soluble Carbohydrates (WSC) determined as per MAFF (1981). Mineral analysis was conducted by a commercial laboratory for Nitrogen (N), Potassium (K), Phosphorus (P), Calcium (Ca), Magnesium (Mg), Sodium (Na), Sulphur (S), Copper (Cu), Cobalt (Co), Iodine (I), Zinc (Zn), Manganese (Mn), Molybdenum (Mo), Iron (Fe) and Selenium (Se), as a percentage on a DM basis, on combined samples from four bales. The Anderson-Darling test was used to test for normality and results were then subjected to a General Linear Model Analysis of Variance and Tukeys HSD post hoc tests.

Results The number of RP decreased as time of soaking increased (t0 > t30, t60 > t80 > t90), as in Table 1. The number of RP decreased as time of steaming increased (t0, t30 > t60, t80 > t90) as in Table 1. Neither steaming nor soaking for any time period significantly affected the WSC of the hay (p>0.05), although greater losses were observed after soaking. Loss in minerals did differ between soaking and steaming treatments, with those minerals that differed significantly shown in Table 1. The time of treatment caused no significant effect on any mineral.

Table 1 The effects of soaking and steaming hay (* p<0.05, ** p<0.01, *** p<0.001)

Treatment time	Respirable Particles (% reduction from control)		Phosphorus* (% DM)		Potassium* (% DM)		Sodium* (% DM)		Sulphur* (% DM)	
	Soak***	Steam***	Soak	Steam	Soak	Steam	Soak	Steam	Soak	Steam
0	0	0	0.28		2.18		0.31		0.17	
30	17	9	0.24	0.27	1.2	2.19	0.21	0.31	0.13	0.18
60	25	24	0.23	0.28	0.96	2.21	0.18	0.34	0.10	0.18
80	41	37	0.25	0.26	0.91	2.15	0.18	0.32	0.12	0.17
90	58	44	0.21	0.25	0.8	2.02	0.18	0.31	0.09	0.17

Conclusion Soaking hay appears to be more effective in reducing respirable particle numbers than steaming, however both methods did significantly reduce numbers of respirable particles. As soaking led to a marked reduction in the nutrient levels of the hay, steaming was preferable to maintain the quality of the hay as a feedstuff, as suggested by Blackmore and Moore-Colyer (1998). Therefore taking all these issues into account steaming hay significantly reduced the number of respirable particles whilst maintaining hay quality. Increasing steaming time to 90 minutes did significantly reduce the number of respirable particles further, however led to a small reduction in the nutrient levels of the hay. There are now several commercially available steamers to steam hay for horses, however their efficacy has not been reported. As there are very few studies investigating steaming hay it is recommended that further work is undertaken in this area and that the efficacy of the commercial steamers be investigated.

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The effect of soluble non-starch polysaccharides in piglet diets on whole digesta viscosity measurements

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Introduction The presence of soluble non-starch polysaccharides (S_{NSP}) in non-ruminant diets can have a negative effect on digestibility due to the increase in digesta viscosity they invariably promote. Viscosity measurements studies have relied on supernatant viscosity following the removal of solid particles from the digesta via centrifugation. However, Takahashi and Sakata (2004) observed that the removal of solid particles from caecal contents changes the basic rheological characteristics of digesta from a non-Newtonian fluid to a Newtonian fluid. The aim of the current study was to investigate the effect of a S_{NSP} source in piglet diets on whole digesta viscosity measurements as determined through a novel protocol developed at Nottingham.

Materials and methods Eighteen piglets weaned at 28 days of age were individually housed and fed one of two diets containing either 150g/kg (D_{SBP}) or 0g/kg ($D_{SBP-free}$) sugar beet pulp (SBP). The experimental period was for 14 days. At slaughter, digesta samples from the first (S_1) and second (S_2) half of the small intestine and caecum (S_3) were taken. Whole digesta viscosity was measured using the Rapid Visco Analyser (RVA) at 960 rpm for 30 seconds followed by 160 rpm for 5 minutes at 37°C. The average viscosity of the last 30 seconds of measurements was taken. Data were subjected to analysis of variance using Genstat 8 and the model was a 2 (Diet) * 3 (Region) factorial.

Results There was a significant effect of diet ($P=0.001$) on whole digesta viscosity, with D_{SBP} being the more viscous in both sections of the small intestine (S_1 and S_2). Generally, there was a significant increase ($P<0.001$) in digesta viscosity for all piglets as the digesta moved down the small intestinal tract; possibly due to the increase in solubilisation and swelling of SBP particles in the diet related to the high water holding capacity (Lee *et al*, 2007). The degradation of caecal microbes and water reabsorption in S_3 can affect the viscosity measurement. The interaction between sites and diets was highly significant ($P=0.006$).

Table 1 The relationship of mean RVA viscosity (cP) of different diets on gastrointestinal regions (S)

	Gastrointestinal Region			ANOVA		
	S_1	S_2	S_3	Factor	s.e.d.	P
D_{SBP}	119	687	970	Diet	84.9	0.001
$D_{SBP-free}$	66	559	267	Site	104.0	<0.001
				Diet*Site	147.1	0.006

Conclusions The results demonstrated differences in whole digesta viscosity measurements of piglets fed differing diets; S_{NSP} such as SBP has a high water holding capacity (Lee *et al*, 2007) leading to the increase of whole digesta viscosity measurements along the small intestine. Whole digesta viscosity could allow better understanding of the effect of S_{NSP} present in non-ruminant diets on the physiological functions of the gut.

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Physical and chemical property of some Iranian non-forage fibre sources

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Introduction Feed by-products are often economical sources of nutrients for use in ruminant rations. However, there are limitations regarding their usage such as high density, small particle size and in some cases, low digestibility due to high lignin contents (for by-products such as cottonseed hull, peanut hull and sunflower hull). The use of some by-products continues to increase (such as pistachio hull and cotton seed hull) although there is little data regarding their physical or chemical properties. In this study some chemical and physical properties of non-forage fibre by-products were investigated.

Materials and methods The six feedstuffs investigated were soy hull, with low seed content (HSH) soy hull with high seed content (LSH), cotton seed hull (CSH), pistachio hull (PH), ground peanut hull (GPH) and sunflower hull (SFH). SFH was manually separated from the seed and LSH was separated from seed by winnowing in the laboratory. PH was cleaned from leaf and stalk manually. Chemical composition was determined by standard methods (AOAC). NDF, ADF and ADL were measured according to Van Soest *et al.* (1991). Initial pH (ipH) and buffering capacity (BC) of feed samples were determined according to Le Ruyet *et al.* (1992). Bulk density (BD), water holding capacity (WHC), soluble ash (solAsh) and soluble DM (solDM) were analysed according to Giger-Reverdin (2000). The physical characteristics data were statistically analysed using the GLM procedure of SAS (V. 9.0). Relationships between chemical and physical parameter variables were investigated using the CORR or REG procedures of SAS.

Results Physical parameters of feedstuffs are shown in Table 1.

Table 1 Physical parameters, initial pH (ipH) and buffering capacity (BC) of Iranian non-forage fibre sources

	BD	WHC (l Kg ⁻¹ DM)	SolDM (% per DM)	SolDM (g/l)	SolAsh (% per ash)	SolAsh (g/l)	ipH	BC (meq/l)
HSH	0.388 ^c	3.32 ^b	31.5 ^b	3.15 ^b	56.00 ^{bdc}	0.345 ^b	6.78	2.07 ^{cb}
LSH	0.418 ^b	4.80 ^a	18.25 ^c	1.83 ^d	62.50 ^{bc}	0.270 ^c	6.81	2.88 ^{cb}
CSH	0.243 ^f	2.73 ^b	11.25 ^d	1.13 ^c	52.00 ^{dc}	0.080 ^d	6.82	1.08 ^{ef}
PH	0.557 ^a	4.53 ^a	52.5 ^a	5.25 ^a	88.00 ^a	1.060 ^a	4.91	7.07 ^a
GPH	0.278 ^c	3.43 ^b	7.75 ^c	0.78 ^c	56.00 ^{bdc}	0.125 ^d	8.28	1.46 ^{ef}
SFH	0.298 ^d	3.54 ^b	7.00 ^c	0.70 ^c	29.00 ^e	0.085 ^d	6.68	0.76 ^f
SEM	0.002	0.095	0.594	0.059	0.058	0.018	-	0.341

BD was higher for PH followed by SH (0.557 and 0.418, respectively). WHC was higher in LSH and PH (4.80 and 4.53l/kg DM, respectively). PH had more SolAsh (5.25g/l), BC (7.07 meq/l) and lower pH (4.91) compare with other sources. PH had higher ash (120.9g/kg DM) non-fibre carbohydrate (NFC, 401.7g/kg DM) contents than the non-forage sources. ADL contents of GPH, CSH and SFH were high (355.5, 209.5 and 200.5g/kg DM, respectively). BD had a high correlation with NDF ($BD=0.654-0.434NDF$, $r^2=0.91$). Also a high correlation between WHC and chemical properties was found with NFC ($WHC=3.12+8.16NFC$, $r^2=0.66$). NDF explained only 0.34 of variation in WHC ($WHC=6.46-3.24NDF$).

Conclusions The observed negative relationship between BD and NDF content of samples (Figure 1) was in agreement with work of Giger-Reverdin (2000). On the other hand, we found a negative relationship between WHC and NDF content of feedstuffs, while Giger-Reverdin (2000) found a positive relationship. This discrepancy might be because of type of feedstuffs used in this work (n=6), whereas Giger-Reverdin (2000) studied a wider range of feedstuffs (n=24). There was no relationship between SolOM and SolAsh. These results indicate feedstuff type can be an important factor in relating their chemical and physical properties.

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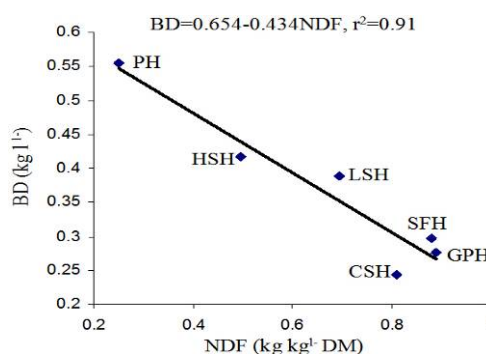


Figure 1 Relationship between BD and NDF.

Nutrient composition in three varieties of full-fat canola seed from Iran

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Introduction During the past 20 years, canola has passed peanut, sunflower and, most recently, cottonseed in worldwide production. At present, some varieties of canola seed are cultivated in Iran particularly Okapi, Opera and SLM046 in east Azerbaijan province. Its components have been altered through genetic selection which markedly reduced its detrimental components. (Leeson and Summers, 2001). There is growing interest within the feed industry to use full-fat oilseeds in poultry diets. There are not enough data of nutrient composition of full-fat canola seed in the country. So the objective of this study was determining of nutrient composition consist of chemical Compounds, mineral content and gross energy in three varieties of canola seed from Iran.

Materials and methods Dry matter (DM), Crude protein (CP), Ether extract (EE), Ash in Samples of grounded Canola seeds were measured according to the method of Association of Official Analytical Chemists (AOAC, 2005). Five samples per treatment were taken. Gross energy (GE) was determined by using an adiabatic calorimetric parr bomb. Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were determined by using a FOSS 1010 Fibertec system. Canola seed samples were analyzed with Flame photometry to determine Na and K contents, with Spectrophotometry to determine P content, with Titration to determine Ca and Mg contents and with Atomic absorption to determine Fe, Mn, Cu, Zn contents. The data was subjected to statistical analysis by univariate procedure of SAS (2002).

Results Table 1 shows the nutrient composition of three varieties of full-fat canola seed. Five samples per each treatment were taken. CP, EE and GE contents were ranged from 19.5%-21.4%, 47.3%-50.4% and 26.779-28.546 Mj/kg in the canola seed varieties respectively. Mean CP content obtained here was less (20.36% vs 23.3%, 25.6%) than that reported by Golian *et al* (2007) and Najib *et al* (2004) respectively. GE and EE contents with averages of 27.451 Mj/kg and 48.77% was considerably higher (27.451 Mj/kg vs 23.551, 17.283 Mj/kg, 48.77% vs 22.7%, 38.2%) than that reported by Golian *et al* (2007) and Najib *et al* (2004). Mean Ca and P contents were result 1% and 0.028% at this study. Ca and P content were reported 0.38% and 0.38% by Najib *et al* (2004).

Table 1 Chemical composition and mineral content of three varieties of Iranian full-fat canola seed.

Items	Canola seed			mean	CV	SD
	Okapi	Opera	SLM046			
DM %	95.1	94.9	93.9	94.63	0.15	0.64
CP%	21.4	20.2	19.5	20.36	4.17	0.85
EE%	47.3	48.6	50.4	48.77	1.89	0.92
Ash%	0.95	0.97	0.95	0.96	1.04	0.01
ADF%	18.5	16.8	18.4	17.9	6.70	1.20
NDF%	19.3	15.7	18.8	17.93	14.16	2.54
GE Mj/kg	28.546	26.779	27.030	27.451	3.46	0.95
Ca%	1.05	0.95	0.7	0.9	7.78	0.07
Mg%	0.13	0.14	0.15	0.14	5	0.007
P%	0.027	0.029	0.028	0.028	3.57	0.001
K %	1.24	1.17	1.16	1.19	4.11	0.049
Na%	0.0125	0.0157	0.0118	0.013	15.38	0.002
Fe mg/kg	51.7	45.2	51.1	49.33	9.30	4.59
Mn mg/kg	35.9	36.7	34.1	35.57	1.60	0.57
Zn mg/kg	11.1	17.6	13.7	14.13	32.48	4.59
Cu mg/kg	12.4	11.8	11.7	11.97	3.5	0.42

Conclusion The results showed that the nutrient composition of canola seeds are considerably different from those which have been reported by former studies, so the present data here on canola seed are recommend in applied animal feeding.

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Determination of the adhesion of enteropathogens to food and feed ingredients

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Introduction Bacterial adherence to host tissues is regarded as an important initial step for colonisation and infection. Hence, agents that interfere with the ability of pathogens to adhere to host cells are promising antidotes. In anti-adhesion therapy, alternative adhesion matrices are orally supplied to competitively bind enteropathogens, thus preventing colonisation and disease. A miniaturised adhesion test (Becker *et al.*, 2007) was applied to analyse the binding capacity of food and feed ingredients for bacterial cells. Different by-products of plant processing were tested in terms of their binding capacity for bacteria in the framework of the EU-project SAFEWASTES, with the aim of turning organic waste into healthy feed. In addition, mannose-containing plant materials such as konjac and locust bean gum were tested, for the most common binding ability described with bacteria up to now is mannose-directed adhesion.

Material and methods Different bacterial strains were allowed to adhere to fibrous materials (Table 1) supplied as well coatings within microtitration plates (Becker *et al.*, 2007). The amount of bacteria retained on the materials was determined in an automated way as growth after addition of BHI or Minca-IsoVitaleX broth. The test principle is based on an inverse relationship between initial cell densities and the appearance of growth: the higher adhering cell numbers the shorter the detection times of growth. The growth curves obtained were fitted by non-linear regression analysis employing a sigmoidal curve model (Becker *et al.*, 2007). Amongst other parameters, detection times $t_{OD=0.05}$ of growth, i.e. the time-coordinate corresponding to an OD_{650nm} -coordinate of 0.05 in each growth curve, were determined from the fitted curves. Detection time means were compared by Fisher's unprotected least significant difference test.

Results Table 1 shows the performance of different materials in terms of their adhesive capacity for different *E. coli* and *Salmonella*.

Table 1 Detection times of growth $t_{OD=0.05}$ [h] of different strains of *E. coli* and *Salmonella*. The data represent least squared means. Materials with the shortest detection time bound most bacterial cells.

Materials	<i>E. coli</i> K88 CIBC 1000	<i>E. coli</i> K99 CIBC 10	<i>E. coli</i> ATCC 25922	<i>S. enterica</i> sv. Thyphimurium RIVM 1287	<i>S. enterica</i> sv. Thyphimurium ATCC 13311	<i>S. enterica</i> sv. Enteritidis ATCC 13076
Artichoke pomace, aerial parts	9.51 ^c	7.49 ^a	2.86 ^b	3.68 ^b	3.97 ^b	4.64 ^{b-d}
BSA (reference)	10.38 ^{d,e}	8.70 ^c	5.14 ^g	3.95 ^d	5.33 ^f	4.58 ^b
Carrot pomace	10.43 ^{d,e}	9.13 ^d	3.23 ^d	4.13 ^e	4.48 ^e	4.82 ^{c-e}
Konjac gum	10.81 ^e	9.19 ^d	2.90 ^c	3.41 ^a	3.56 ^a	3.81 ^a
Locust bean gum	9.93 ^{c,d}	8.63 ^c	3.51 ^f	4.92 ^f	5.40 ^f	5.00 ^{e-f}
Palm kernel meal	8.72 ^b	8.31 ^b	3.38 ^e	3.34 ^a	3.79 ^b	4.63 ^{b,c}
Pumpkin pulp and peel	7.48 ^a	8.45 ^c	2.82 ^{a,b}	3.63 ^b	4.24 ^c	5.13 ^{f-g}
Sesame seed meal	7.74 ^a	8.19 ^b	2.91 ^c	3.40 ^a	3.80 ^b	4.57 ^b
Tempeh (fermented soya bean)	7.20 ^a	8.56 ^c	2.76 ^a	3.57 ^b	4.18 ^c	4.88 ^{d-e}
Tomato, whole fruit	7.79 ^a	8.25 ^b	2.78 ^{a,b}	3.68 ^b	4.24 ^c	5.28 ^g

Conclusions With growth as measuring variable for adhesion, a simple high-throughput method was applied for the screening of large numbers of different binding matrices and bacteria. The *in vitro* test proved highly sensitive and discriminating for different materials (Table 1). The respective differences in the capacities of natural materials to bind different strains underline the importance of extensive testing. In a follow-up study, *in vivo* tests with challenged animals have to be performed with promising materials to validate the anti-adhesion principle in the complex system of living beings.

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The degradation kinetic of NDF and lignin of sunflower meal containing low or high fat treated with sodium hydroxide

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Introduction Sunflower meal is a good source of protein and NDF for ruminants. The amount of hull or fibre in sunflower might affect the ruminal and post-ruminal digestion of this protein source. Available information on the effect of sodium hydroxide on degradation of NDF and lignin of sunflower meal is scarce. It has been proposed that sodium hydroxide may break down hemicellulose, hydrolyze the ester bonds between lignin and hemicellulose, swell cellulose microfibrils (Canale *et al.*, 1992), expose the cellulose to microbial attachment and improve digestibility (Goto *et al.*, 1993). The objective of this study was to investigate the effect of sodium hydroxide (NaOH) on chemical composition and *in situ* degradation characteristics of NDF and lignin of low and high fat sunflower meal (25 and 165 g/kg DM; LFSM and HFSM, respectively).

Materials and methods Experimental samples were: 1- Untreated LFSM (ULFSM), 2- NaOH treated LFSM (40 g/kg, DM, LFSM+SH); 3- Untreated HFSM (UHFSM) and 4- NaOH treated HFSM (40 g/kg, DM, HFSM+SH). Chemical composition (CP, Ash, lignin, NDF and ADF-ash) was determined using standard procedures (AOAC, 1990). Lignin and NDF degradation of the samples were determined using *in situ* technique in two fistulated Holstein steers (400±12 Kg, body weight). Animals were fed 8.8 kg of DM of a diet consisted of 40% concentrate (155 g CP kg⁻¹ of DM; 30% maize, 34% barley, 8% soybean meal, 5% sugar beet pulp, 10% wheat bran, 12% cottonseed meal, 0.3% CaCO₃, 0.5% mineral and vitamin premix, 0.2% salt), 30% lucerne hay and 30% maize silage. Samples were ground to pass through a 2-mm screen and placed (5 g DM) in polyester bags (10×20 cm, 50 µm pore size), then, incubated in the rumen for 0.0, 2, 4, 6, 8, 16, 24, 48, 72 and 96 h (n=8). Data of NDF and lignin degradation beyond the lag-time were further adjusted to a negative exponential model of $P = a + b(1 - e^{-ct})$, where P= fraction degraded in the time t, a= rapidly degradable fraction, b= slowly degradable fraction, c= fractional degradation rate and t= incubation time. Data were analyzed using general linear model of SAS (1990) at P< 0.05.

Results Chemical composition of the samples is summarized in Table 1. Ruminal degradation parameters (a, b, c) of NDF and lignin are shown in Table 2. NaOH caused to increase fraction (b) of NDF of sunflower meals (low and high fat). The (b) fraction of lignin was also improved when samples were treated with sodium hydroxide.

Table 1 Chemical composition (g/kg DM) of sodium hydroxide treated sunflower meals

Chemical composition	Treatments*				s.e.m
	ULFSM	LFSM+SH	UHFSM	HFSM+SH	
CP	337.6 ^a	305.1 ^b	278 ^c	234.6 ^d	1.0
Ash	59.9 ^c	99.2 ^a	49.6 ^d	80.1 ^b	0.4
NDF	400 ^b	410 ^b	400 ^b	430 ^a	14
Lignin	93 ^b	126 ^a	83 ^b	130 ^a	6
ADF-ash	330 ^a	276 ^b	323 ^a	321 ^a	7

*Untreated LFSM (ULFSM); NaOH treated LFSM (40 g/kg, DM, LFSM+SH); Untreated HFSM (UHFSM); NaOH treated HFSM (40 g/kg, DM, HFSM+SH); s.e.m: Standard error of mean, Means with different letters within samples differed (P< 0.05)

Table 2 *In situ* NDF and lignin (mean ± SE) degradation coefficients (a, b, c) of sunflower meals

	NDF				Lignin			
	ULFSM	LFSM+SH	UHFSM	HFSM+SH	ULFSM	LFSM+SH	UHFSM	HFSM+SH
a	0.13±0.02	0.12±0.01	0.13±0.01	0.11±0.01	0.06±0.01	0.05±0.01	0.06±0.01	0.06±0.01
b	0.72±0.15	0.79±0.20	0.45±0.10	0.54±0.10	0.27±0.80	0.80±0.64	0.26±0.52	0.79±0.88
c	0.02±0.01	0.01±0.04	0.07±0.03	0.04±0.04	0.00±0.01	0.00±0.01	0.01±0.01	0.00±0.04

a: Rapidly degradable fraction; b: Slowly degradable fraction; c: Fractional degradation rate constant (h⁻¹)

Conclusions Results of the present experiment indicated that the CP concentration of samples containing both fat were significantly decreased when they treated with NaOH (P< 0.05). Slowly degradable fraction (b) of NDF of ULFSM was higher than UHFSM, while, there was not difference in fraction (b) of lignin. It was concluded that NaOH caused to increase the degradation potential of sunflower meal. In addition, the potential degradation of NDF and lignin of sunflower meal might be affected by the fat concentration.

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Effects of *Lactobacillus plantarum* MTD1 on fermentation and nutritive value of low dry matter maize silage

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Introduction In Iran, maize silage is used in dairy cattle farm as a forage source and harvested as a second crop, so has a high moisture (about 800 g/kg). If used as silage, it is susceptible nutrient loss as effluent, and clostridium bacteria growth so that a poor quality silage results. In order to improve the ensiling process, biological additives have been developed. These additives reduce the dependence of the ensiling process on epiphytic lactic acid bacteria (LAB). The function of these inoculants is to ensure a rapid and efficient fermentation of water soluble carbohydrate (WSC) into lactic acid. Most available inoculants consist of selected strains of homo-fermentative LAB, such as *Lactobacillus plantarum*, *Pediococcus* and *Enterococcus* species. Many studies have shown the advantages of LAB inoculants. The purpose of this study was to focus on the effects of *Lactobacillus plantarum* on the fermentation characteristics and *in vitro* dry matter digestibility (IVDMD) of low dry matter (DM) maize silages.

Materials and methods Maize forage (hybrid 700, used extensively in Iran) was harvested at the milk stage (228 ±9.0 g/kg DM) and chopped to about 2.5 cm, treated with inoculants and ensiled in 3.5 l glass jars equipped with a lid that enabled gas release. In this experiment, 24 jars were allocated to treatments in three replicate on days 6, 16, 21 and 90 after ensiling then opened and sampled for chemical analyses. The following treatments were used, control (no additive) and Inoculant (Ecosyl, UK, containing *L. plantarum* MTD1 about 1×10^5 colony forming units (cfu) g⁻¹ wet forage). Dry matter was determined by oven drying for 48 h at 60°C. Crude protein (CP) was determined by a Kjeldahl method and ammonia-nitrogen (NH₃-N) according to Filya (2003). WSC was determined by the phenol sulphuric acid method and lactic and acetic acids by gas chromatography. IVDMD was measured according to Tilley and Terry (1963). Silage data were analyzed using the GLM procedure of SAS. Models included days of ensiling, and inoculant as main effects, and days of ensiling × inoculant, as interactions for silage analysis data.

Results In this investigation, DM, CP, NDF, Ammonia-N and Lactic acid weren't affected by inoculation, but pH, WSC, Acetic acid, and IVDMD content were affected by inoculation. Overall inoculated silages had a higher pH and lower IVDMD, WSC and acetic acid than control silages. During fermentation pH and WSC significantly were decreased, but lactic acid and ammonia-N significantly and acetic acid numerically increased (Table 1).

Table 1 Effect of *Lactobacillus plantarum* and days on fermentation and nutritive value of maize silage (g/kg DM)

Treatments Parameters	Inoculation		Days				s. e. d.	P value		D*I
	Un	In	6	16	21	90		In	Day	
DM	206.5	213.3	206.1	210.4	205.8	214.0	2.056	0.078	0.116	0.448
pH	3.74 ^b	3.81 ^a	3.95 ^a	3.76 ^b	3.64 ^c	3.76 ^b	0.026	0.033	0.001	0.428
CP	74.5	79.0	76.55	78.6	79.17	73.35	0.858	0.137	0.242	0.576
NDF	486	502	493.2	511.5	488.3	499.5	4.653	0.064	0.409	0.655
Lactic acid	59.25	65.96	40.26 ^b	62.4 ^a	72.8 ^a	74.8 ^a	4.122	0.272	0.002	0.161
Acetic acid	10.03 ^a	5.39 ^b	4.57	8.07	8.31	9.89	0.876	0.003	0.083	0.577
NH ₃ -N	2.85	2.70	1.95 ^b	2.74 ^a	3.02 ^a	3.41 ^a	0.157	0.573	0.007	0.783
WSC	52.47 ^a	37.3 ^b	50.89	40.98	41.33	50.89	2.680	0.006	0.429	0.988
IVDMD	557.4 ^a	513.6 ^b	539.5	548.8	526.8	526.8	8.163	0.010	0.688	0.359

Un = Uninoculated, In = Inoculated. (D×I) = Interaction effects, NH₃-N = ammonia-nitrogen, a and b show the comparison of treatments.

Conclusions In this experiment, both control and inoculated of silages were well preserved as indicated by low pH and ammonia-N concentration. This referred to extensive fermentation of maize silage with low DM. The lack of response to inoculation can be attributed to the high number of lactic acid bacteria which were present on the maize crop prior to ensiling. In conclusion, the results of this investigation showed that homo-fermentative LAB inoculants cannot improve the fermentation parameters of low DM maize silage more than what epiphytic lactic acid bacteria that are able to do.

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Effect of defaunating the rumen of sheep fed various diets on gas production *in vitro*

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Introduction Although roughages are the main components of diets for ruminants their endogenous enzymes cannot digest lignocellulosic feedstuffs but is carried out by the rumen microbial population which comprises of bacteria, protozoa and fungi. Their main function in the rumen is the fermentation of feeds which yields energy to the host animal in the form of short chain fatty acids. Recent interest in the efficient utilisation of roughage diets has led to an increase in the use of the gas production (GP) technique due to the advantage of studying fermentation kinetics. This technique provides useful data on digestion kinetics of both soluble and insoluble fractions of feedstuffs. Several GP approaches have been developed including the use of glass syringes (Menke *et al.*, 1979; Blümmel *et al.*, 1997) which appears to be the most suitable for use in developing countries. The objective of this experiment was to evaluate the effect of defaunating the rumen of sheep on the amount of GP from wheat straw, alfalfa hay, cottonseed meal and barley grain.

Materials and methods Four rumen-cannulated sheep (Shaal breed) were used and fed four different mixed diets containing 0, 20, 40 or 60% concentrate across two stages; faunated and defaunated rumen. Defaunating the rumen was done using the emptying and rumen washing technique of Jouany and Senaud (1979). Gas production was measured using the method of Menke *et al.* (1979) with fermentations conducted in 100 ml calibrated glass syringes containing 200 mg feed dry matter (DM) and buffered rumen fluid. Incubation was carried out in an incubator set at 39°C for 24 and 96 h. The levels of other chemical constituents were also determined. The study was completed as a Latin square design and the GP data analysed for the main effects using Minitab®. All multiple comparisons between means were performed using Duncan's multiple range test.

Results The GP results for the feeds studied are given in Table 1 and indicated that there was no significant effect of concentrate level in the basal diet on GP to 24 or 96 h for the feeds when incubated with rumen fluid from either faunated or defaunated animals. Defaunation resulted in a significant reduction in GP at 24 h for wheat straw (33.3 vs 43.3 ml) after 24 incubation in rumen liquor ($P < 0.05$).

Table 1 Amount of gas produced (ml) after 24 and 96 hr incubation from wheat straw, alfalfa hay, cottonseed meal and barley grain using rumen fluid faunated and defaunated animals

		Feed stuffs								
		Wheat straw		Alfalfa hay		Cottonseed meal		Barley grain		SEM/P
Condition	Diet/time	24	96	24	96	24	96	24	96	
faunated	0% conc.	43.5	59.3	46.3	56.3	38.5	53.3	66.3	89.5	2.10/0.01
	20% conc.	44.0	59.0	46.5	55.0	32.8	50.5	65.0	90.5	1.90/0.01
	40% conc.	44.7	59.5	47.3	55.5	35.0	53.8	71.8	89.5	2.25/0.01
	60% conc.	44.3	58.0	46.3	55.5	34.8	52.3	77.0	90.3	2.35/0.01
	Mean ± SD	44.3 ^a ± 2.68	58.9± 1.48	46.4± 2.39	55.56± 1.97	35.3± 3.68	52.3± 4.58	70.0± 9.04	89.9± 3.44	
defaunated	0% conc.	30.7	51.5	40.0	50.0	34.8	54.5	65.3	84.3	2.30/0.01
	20% conc.	32.0	53.3	37.8	47.0	36.5	53.3	64.5	87.8	2.15/0.01
	40% conc.	34.0	53.5	41.3	49.8	32.5	48.5	64.5	93.5	2.25/0.01
	60% conc.	36.7	62.0	62.0	44.0	34.8	44.8	62.5	66.8	1.85/0.01
	Mean ± SD	33.3 ^b ± 5.7	55.1± 5.57	40.7± 4.58	50.5± 5.42	34.6± 2.11	54.7± 10.7	65.3± 1.08	90.8± 10.5	

Conclusions By defaunating the rumen the volume of gas produced decreased (except for cottonseed meal) and significant differences ($p < 0.05$) were observed for wheat straw.

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Study of steam pressure and reaction time effect on chemical composition and degradability of palm date leaves

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Introduction Palm date leaves is an agricultural by product with high levels of lignocellulosic materials that limite its bioavailability for microbial and gastrointestinal tract enzymes. It contains more than 40 percent cellulose and 20 percent hemicellulose. The nature of steam treatment was acid hydrolysis which effectively affected cell wall fractions (Castro *et al*, 1993). Production of palm date leaves in Iran is more than 500,000 ton annually. The aim of this study was to assess the effects of steam pressure and reaction time on chemical composition and bioavailability of palm date leaves to rumen microbes.

Material and methods sample (700 g)containing 50% moisture were collected from palm groves of Khoozestan and were put in stainless steel baskets and placed in reaction chamber. Steam treatment of samples carried out by direct injection of steam in to the chamber and samples kept under a specific pressure and a time(reaction time) and them the chamber were depressurized. A complete randomized design with a 3×3 factorial arrangement.(three pressures including 1.4×10^3 , 1.7×10^3 and 2.0×10^3 pa. and 3 reaction times including 120, 180 and 240 seconds) with three replicates was used to assess the effects of pressure and reaction time on chemical composition , *in situ* degradation(Mehrez and Orskov, 1977) and *in vitro* gas production (GP)(Menke and Steingass, 1987) of samples. Dry matter loss(DML) of samples during the treatment was determined bye measuring the changes in dry matter of the samples before and after treatment. Samples were analyzed for water soluble sugars (WSS) (Dubois *et al*, 1956), NDF, hemicellulose(HEM)(Ternud *et al*, 1989) ,total phenolics content(Tph) (Julkunen- Tiitto, 1985).

Results Both pressure and reaction time significantly affected ($p<0.05$) the chemical composition and bioavailability of palm date leaves (Table 1) and an interaction was also observed ($p<0.05$) between pressure and reaction time. DML and WSS contents of samples increased with elevating pressure and reaction time. Changes of HEM and NDF were parallel , also results showed that steam treatment improved gas production and degradability

Table 1 chemical composition *in vitro* and *in situ* degradability of steam treated palm date leaves

Pressure (pa)	Reaction time(s)	% DML *	% WSS	%NDF	% HEM	%Tph	GP 24h(ml)	%Deg.48h (<i>in situ</i>)
Untreated samples		-	1.40	71.07	26.46	15.45	14.74	38.71
1.4×10^3	120	1.82 ^d	6.51 ^c	66.97 ^a	19.75 ^a	27.08 ^a	21.20 ^d	54.27 ^d
	180	2.23 ^d	7.19 ^d	64.6 ^b	19.8 ^a	25.02 ^{ab}	22.72 ^c	58.48 ^c
	240	2.62 ^d	8.12 ^c	63.47 ^{bc}	18.73 ^a	23.79 ^{abc}	23.82 ^{bc}	59.38 ^c
1.7×10^3	120	3.88 ^c	7.95 ^c	61.97 ^c	19.03 ^a	24.13 ^{abc}	23.99 ^b	59.20 ^c
	180	3.99 ^c	8.89 ^b	62.4 ^c	16.22 ^b	22.76 ^{abcd}	25.84 ^a	62.95 ^b
	240	4.37 ^{bc}	9.75 ^a	59.37 ^d	14.83 ^b	19.49 ^{cd}	26.26 ^a	63.27 ^b
2.0×10^3	120	4.88 ^b	9.17 ^b	58.27 ^d	15.9 ^b	21.82 ^{bcd}	26.26 ^a	62.64 ^b
	180	6.22 ^a	10.02 ^a	54.77 ^e	11.42 ^c	20.54 ^{bcd}	26.72 ^a	68.82 ^a
	240	6.38 ^a	9.24 ^b	55.57 ^e	8.73 ^d	18.82 ^d	26.35 ^a	68.67 ^a
SEM(n=3)		0.44	0.185	1.97	2.99	4.34	0.91	2.31

*DML: Dry Matter Loss, WSS: Water Soluble Sugars, NDF: Neutral Detergent Fiber , HEM: Hemicellulose, Tph: Total phenolics content, GP 24h(ml): Gas Production after 24 hour incubation, Deg.48h: Degradability after 48 hour incubation.

-Means on the same column with different letters are significantly differ($p<0.05$)

Conclusion steam treatment effectively improved value of palm date leaves. steam treatment hydrolysed the hemicellulose to water soluble sugars and also depolymerization of lignin during the treatment increased the bioavailability of cell wall content as gas production and *in situ* degradability . But changes in Tph content of samples did not follow the same pattern as NDF and HEM which was very likely due to contribution of some artifacts from degradation of carbohydrate to artifacte lignin(Chua and Wayman,1978). Dry matter loss increased with increasing of steam pressure and reaction time and can decrease nutritive value of substrate. Treatment with steam pressure in 2.0×10^3 pa. and 180 second had the best result in more items, longer reaction times had negative effect on dry matter loss. Thus higher pressure and shorter reaction time is need to achieve the better nutritive value.

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Effects of steam treatment on the enzymic hydrolysis and bioutilisation of palm date leaves

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Introduction The bioavailability of palm date leaves is low because of high levels of lignocellulosic materials in the cell wall. The presence of lignin protects carbohydrates from attack by rumen microbes (De La Cruz,1990). Steam treatment increases feed intake, overall digestibility and live weight gain, because of the extensive destruction of the cell wall (Castro and Machado,1990). Steam treatment effects have been ascribed to complete hydrolysis of hemicellulose (Grohmann *et al.*, 1985), lignin depolymerization and redistribution within the cell wall (Toussaint *et al.*, 1991). The aim of this study was to assess the effect of steam pressure and reaction time on bioutilisation of palm date leaves.

Material and methods Samples containing 50% moisture were put in stainless steel baskets and placed in the reaction chamber. Steam treatment of samples was carried out by direct injection of steam into the chamber and samples were kept under a specific pressure and time(reaction time) before the chamber was depressurised. A complete randomised design with a 3×3 factorial arrangement (three pressures, 14, 17 and 20 atm. and three reaction times, 120, 180 and 240 s) with three replicates was used to assess the effects of pressure and reaction time on Accessible Pore Volumes (APV) (Stone and Scallan, 1968) and enzyme hydrolysis (Nelson, 1944). APV for 8, 12, 51, 110, 270 and 550 Å probe molecules were measured according to unabsorbed sugar concentration of samples. After characterisation of optimum enzyme/substrate ratio, samples were incubated with 64 IU cellulose Onozuka (EC: 3.2.1.4) for 48 h and then the reducing sugar content was determined. Data were analysed (ANOVA) and means were tested according to Duncans's least range test with all means being compared with untreated substrate (control) by Dunnett's test.

Results Results are presented in Table 1 which shows that steam pressure treatment significantly increased reducing sugar concentration after enzyme hydrolysis and also values of APV were improved compared with untreated palm date leaves ($p<0.05$). Increasing pressure and reaction time resulted in considerable improvement of parameters but interaction of steam pressure and reaction time were not significant. Changes of residual sugar concentrations were similar to changes of APV.

Table 1 Reducing sugar content and APV of steam treated palm date leaves

Pressure (atm)	Reaction time(s)	R.Sug*	APV ₈ (ml)	APV ₁₂ (ml)	APV ₅₁ (ml)	APV ₁₁₀ (ml)	APV ₂₇₀ (ml)
Untreated samples		6.03	1.21	0.46	0.29	0.2	0.07
	120	7.11 ^c	1.53 ^c	0.7 ^f	0.54 ^f	0.34 ^h	0.16 ^f
14	180	7.79 ^{bc}	1.35 ^d	0.76 ^e	0.56 ^{ef}	0.37 ^g	0.17 ^f
	240	8.40 ^b	1.59 ^{bc}	0.83 ^d	0.59 ^{de}	0.42 ^f	0.19 ^c
	120	8.36 ^b	1.51 ^c	0.91 ^c	0.63 ^d	0.52 ^c	0.20 ^{de}
17	180	9.6 ^a	1.53 ^c	0.94 ^c	0.63 ^d	0.57 ^d	0.21 ^d
	240	10.21 ^a	1.6 ^{bc}	0.95 ^c	0.69 ^c	0.63 ^c	0.23 ^c
	120	8.18 ^{bc}	1.7 ^{ab}	1.02 ^b	0.72 ^c	0.67 ^b	0.25 ^b
20	180	9.87 ^a	1.75 ^a	1.12 ^a	0.77 ^b	0.70 ^a	0.26 ^{ab}
	240	9.68 ^a	1.76 ^a	1.15 ^a	0.86 ^a	0.71 ^a	0.27 ^a
SEM(n=3)		0.84	0.011	0.002	0.002	0.001	0.0001

* R.Sug: Reducing Sugar%, APV₈, APV₁₂, APV₅₁, APV₁₁₀ and APV₂₇₀ Accessible Pore Volumes for 8,12,51,110 and 270 Å (ml/g).

Means with different letters in same column are significantly different($p<0.05$)

Conclusion Steam treatment of palm date leaves improved its bio utilisation because of cell wall destruction and increased accessible pore volumes which would result in better availability of cell walls for cell free enzymes. Higher pressures require shorter reaction times to achieve the same nutritive value as lower pressures with longer reaction time.

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The effects of bacterial inoculation and hybrids on fermentation characteristics and *in vitro* dry matter digestibility of maize silage

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Introduction Maize silage is a major source of forage for lactating dairy cattle throughout Iran. Therefore, it is important to understand and evaluate the characteristics that affect its feeding value of maize silage. Maize hybrids for ensilage and epiphytic lactic acid bacteria are the factors that can alter the nutritive value of maize silage. Homolactic bacteria have been used to inoculate silage and improve their fermentation and several studies have demonstrated the effects of inoculation of maize silage on silage fermentation characteristics. Hybrid also influenced the chemical characteristics of the maize silage. This experiment was conducted to evaluate the effects of bacterial inoculation and hybrids on fermentation quality and nutritive value of two Iranian maize hybrids.

Materials and methods The experiment was conducted using mini-silos (3.5 l) in a 2 × 2 factorial arrangement. Two hybrids of maize (T.W.C. 647 and S.C.704) were harvested at the milk stage and then chopped to ~2.5 cm lengths by chopper. The additive treatments were control (no additive) and inoculant (Ecosyl, UK, containing *L. plantarum* MTD1) applied at 1 × 10⁵ colony forming units (cfu) g/fresh forage. Each treatment was ensiled in triplicate. Mini silos stored at ambient temperature (18 to 28°C) in a laboratory. Effluent was collected as g per kg fresh material. The mini-silos were opened 120 d after ensiling and a sub-sample was dried at 55°C (72 h) in an oven to determine dry matter (DM) concentration and DM recovery following fermentation. Water soluble carbohydrates (WSC) were determined by the phenol sulphuric acid method and *in vitro* DM disappearance (IVDMD) was obtained using the first stage of the method of Tilley and Terry (1963). Ammonia-nitrogen (NH₃-N) was determined using the method of Filya (2003). Fresh samples of maize silage from each mini-silo were extracted and analysed for pH, using a digital pH meter, and acetate and lactate by gas chromatography. Dried samples were analysed for crude protein (CP; micro auto-Kjeldahl), and neutral detergent fibre (NDF) with sulfite (Van Soest *et al.*, 1991). The statistical analysis was completed as a factorial design (2×2) with two hybrids and two inoculant treatments using ANOVA by PROC GLM of SAS.

Results Hybrid 647 had a significantly (P<0.001) lower and higher DM and CP content respectively than hybrid 704. The NDF, lactate, WSC, NH₃-N concentrations and pH were not significantly affected by hybrids or inoculation, but acetate was significantly affected by hybrid. Inoculation had a small effect on maize silage fermentation characteristics. The inoculated hybrid 704 maize silage had a lower effluent than the untreated control maize silage. The IVDMD was greater in the inoculated hybrid 704 maize silage than other silages. Uninoculated hybrid 647 corn silage had a lower DM recovery than the other silages (Table 1).

Table 1 Effect of hybrids and inoculant on fermentation and IVDMD of maize silage (%DM unless stated otherwise)

Hybrid/ Inoculant	DM (g/kg)	pH	CP	NDF	WSC	IVDMD	Lactic acid	Acetic acid	NH ₃ -N	DM recovery (%)	Effluent (g/kg)
704	C 28.65 ^a	3.76	7.23 ^b	44.88	3.25	54.88 ^b	7.49	1.40	0.35	94.66 ^a	34.94 ^a
	I 29.06 ^a	3.68	6.85 ^b	43.39	2.74	61.62 ^a	7.33	1.19	0.37	93.94 ^a	15.25 ^b
647	C 18.18 ^b	3.73	8.51 ^a	50.39	2.17	55.04 ^b	7.76	1.08	0.33	83.34 ^b	49.97 ^a
	I 19.35 ^b	3.64	8.57 ^a	48.72	2.44	55.66 ^b	8.85	1.54	0.34	94.01 ^a	45.63
p	H 0.001	0.174	0.001	0.244	0.071	0.002	0.178	0.015	0.721	0.003	0.006
	I 0.951	0.396	0.668	0.734	0.391	0.625	0.039	0.754	0.233	0.007	0.764
	Int 0.167	0.080	0.132	0.947	0.558	0.001	0.071	0.261	0.662	0.003	0.268
	se 1.060	0.264	0.18	1.295	0.419	1.042	0.485	0.105	0.013	1.54	5.006

C = Control (Uninoculated), I = Inoculated, H = Hybrid, Int = Hybrid × Inoculant interaction.

Conclusions Overall, genetic variation did not alter the fermentation pattern of maize silage, but significantly affected DM digestibility and DM recovery. Inoculation with lactic acid bacteria did not improve silage fermentation and IVDMD. Inoculation significantly increased IVDMD and decreased the effluent of hybrid 704 maize silage and was attributed to a higher DM. There was a significant interaction for H × I on *in vitro* digestibility that was attributed to the effects of microbial inoculation on low moisture corn silage (hybrid 704) that caused fast pH drop so decreased nutrition losses. Inoculation also increased DM recovery in hybrid 647 maize silage which may be due to the extensive fermentation in the uninoculated hybrid 647 maize silage compared with inoculated one.

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Effect of lucerne hay particle size and physical effectiveness factor of diet on utilisation of soya hulls by dairy cows

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Introduction The effect of inclusion of soya hulls (SH) as grains or forages sources in rations of dairy cows have been studied in many experiments. However, when SH are used to substitute for forage sources they induced milk fat depression, lower rumination time and ruminal pH (Weidner and Grant, 1994a, b). There is no data on effectiveness of diet particle size when SH used as a forages source. The objective of this study was to investigate the effect of physical effectiveness factor (pef) of diet on utilisation of SH in dairy rations.

Material and methods Eight lactating Holstein cows (four preparturient and four multiparous; 590±33 kg of body weight and 47±13 days in milk (DIM)) were used in two latin square design (two square; one for preparturient and one for multiparous cows) with four 19-d periods (last 7-d for data collection) and a 2×2 factorial arrangement of treatments: lucerne hay (LH) particle size (fine; 5mm vs. coarse; 20mm) combined with SH content (none or substituted for 50% of LH; LH and LH+SH, respectively). The basal diet was formulated according to NRC (2001) requirements and consisted of 60% barley-based concentrate and 40% forage with net energy of lactation (NEL) content of 6.57 KJ/kg dry matter (DM) and crude protein, neutral detergent fibre (NDF) and acid detergent fibre contents of 17.1, 31.5 and 19.2 respectively. Diets were iso-energetic/nitrogenous. The SH diet and basal diets contained 47% and 63% respectively of their NDF from forage. The LH level in the basal diet was 13.5% DM. Differences in mean particle length and the pef of the diets were created by manipulating of LH particle size and SH substitution with LH. Rumen fluid sampled at 4 hours after morning feeding and sampled by stomach tube. Faecal samples were taken after morning feeding on days 15 and 17, and mixed with deionised distilled water (1:1). Milk sampled on 13th and 16th days of each period at three consecutive milking and pooled based on production per sampling days and analysed for fat, protein, lactose and solid not fat (SNF) by milk analyzer (Astori, Italy). The statistical model was: $Y_{ij(kl)m} = \mu + SQ_m + \text{period}(SQ)_{im} + \text{Cow}(SQ)_{jm} + A(K) + B(l) + (AB)(kl) + \varepsilon_{ij(kl)m}$, Where $Y_{ij(kl)m}$ = observation $ij(kl)m$; μ = the overall mean; SQ_m = the effect of square (parity); $\text{period}(SQ)_{im}$ = the effect of period i within square m ; $\text{Cow}(SQ)_{jm}$ = the effect of cow j within square m ; $A_{(k)}$ = the effect of level k of factor A; $B_{(l)}$ = the effect of level l of factor B; $(AB)_{kl}$ = the effect of interaction of level k of factor A with level l of factor B and $\varepsilon_{ij(kl)m}$ = random error with mean 0 and variance σ^2 . Data were analyzed using the GLM procedure of SAS (V 9.0).

Results The DM intake, milk production and composition, faecal and rumen pH of cows and pef of diets are shown in Table 1. The main effect of SH on milk production, milk fat and faecal pH was significant. Cows received SH produced more milk ($P < 0.05$) but had lower fat in their milk ($P < 0.01$). Cows received SH had lower faecal pH ($P < 0.01$). Multiparous cows consumed more DM ($P < 0.01$) and produced more milk ($P < 0.04$), had higher faecal pH, lower milk SNF and protein ($P < 0.01$) compared with preparturient cows (data not shown). The effect of particle size (PS) and SH on pef was significant ($P < 0.01$).

Table 1 Means of DMI, milk production and composition, faecal and rumen pH of cows and pef of diets

	Diets				SE	P value		
	LH		LH+SH			PS	SH	PS × SH
	fine	coarse	fine	coarse				
DMI (kg/d)	22.29	21.68	21.99	22.83	0.59	0.84	0.48	0.24
Milk production (kg/d)	34.59	34.24	36.26	35.87	0.71	0.6	0.04	0.98
Fat corrected milk (kg/d)	31.41	31.54	30.467	32.72	0.97	0.24	0.90	0.29
Milk composition								
Fat (%)	3.55 ^a	3.52 ^a	2.95 ^b	3.47 ^a	0.1	0.3	<0.01	0.01
Protein (%)	3.06	3.04	3.05	3.05	0.02	0.45	1.0	0.57
SNF (%)	8.27	8.33	8.33	8.29	<0.01	0.85	0.85	0.519
Lactose (%)	4.58	4.53	4.87	4.43	0.15	0.11	0.59	0.19
Rumen pH	6.27	6.41	6.16	6.33	0.12	0.19	0.41	0.89
Faecal pH	6.26 ^{ab}	6.34 ^a	6.22 ^{ab}	6.11 ^b	0.04	0.75	<0.01	0.04
Particle size distribution								
pef ¹	64.39 ^b	69.39 ^a	69.10 ^a	71.01 ^a	1.09	<0.01	<0.01	0.17

¹ Physical effectiveness factor determined as the proportion of particles retained on 3 sieves of new Penn stat particle separator (Kononoff *et al.*, 2003).

Conclusions The effect of SH on milk production maybe because of more DM digestibility of SH in rumen. Lower faecal pH with SH was only seen when coarse LH was fed. Coarse LH may have induced greater dilution rate and so greater passage rate of SH from the rumen to hind gut. Fine lucerne hay reduced pef of the diet, but substitution of SH with fine lucerne hay increased pef similar to the coarse lucerne ration with or without SH. With the consideration of lower milk fat in animals receiving SH treatments, it can be concluded that SH is not as effective as LH in milk fat production.

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Nutritional evaluation of low dry matter maize silage ensiled with or without a bacterial inoculant: 2. Effects on aerobic stability and animal performance

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Introduction Microbial inoculants are generally used to provide a better fermentation condition in silage making. A decrease in yeast and mould numbers with improvement in aerobic stability of high moisture maize silages has also been reported due to a microbial inoculation (Filya *et al.*, 2004). Changes were observed in milk yield and composition of cows consuming diets containing inoculated silage (Kurtoglu and Coskun, 2003). The objectives of this experiment were to investigate the effects of Lalsil inoculant on aerobic stability of maize silage, and on feed intake, milk yield and composition of Holstein dairy cows when fed inoculated silage.

Materials and methods Maize (*Zea mays* L.) forage was harvested by chopper at a dry matter (DM) of about 220 g/kg and ensiled in 40-Ton capacity silo with or without a bacterial inoculant (Lalsil MSO1, Lallemand, France). Details of the inoculant composition and silage preparation are described previously (Khadem *et al.*, 2008). After two months of the ensilage period, silage samples from each treatment (about 10 kg) were collected and allowed to remain aerobic at room temperature. The ambient and silage temperature were recorded every 2 hours. Aerobic stability was defined as the number of hours the silage remained stable before rising more than 2 °C above the ambient temperature. Twenty two Holstein dairy cows (600 ± 30 kg body weight, 35 ± 6 kg/d milk yield and at 12 ± 4 weeks of lactation) were used for eight weeks in the experiment. The animals were randomly allocated into two treatment groups based on milk yield, calving date and parity and had *ad libitum* access to a TMR diet either with uninoculated or inoculated maize silage after two weeks adaptation period. Diets were formulated according to NRC (2001) and contained concentrate to forage ratio of 65:35 on a DM basis. Cows were milked three times per day at 5.00, 13.00, and 21.00 h and milk volume was recorded at each milking time. Feed intake was measured for each group daily. Milk samples were collected from morning milking for each cow once a week for analysis of fat, protein, lactose, solid not fat contents (SNF) and urinary nitrogen contents. Data were analysed by one-way analysis of variance using Minitab to study if the effects were significant at P<0.05.

Table 1 Dry matter intake (DMI), milk yield and composition of cows fed total mixed diets containing either uninoculated or Lalsil inoculated low dry matter maize silage

Parameters	Treatments		SEM	P value
	Uninoculated silage	Inoculated silage		
DMI (kg/100kg live weight)	3.75	3.79	0.12	ND
Milk yield (kg/d)	33.59	33.93	1.1	0.83
Milk composition (g/kg)				
Fat	37.4	37.8	0.8	0.74
Protein	31.1	31.5	0.5	0.61
Lactose	46.6	48.5	0.6	0.04
Solid not fat	86.4	89.0	0.9	0.05
Milk urinary nitrogen	0.54	0.54	0.02	0.41

DMI, dry matter intake; ND, not determined

Results Upon aerobic exposure, the uninoculated silage spoiled faster with the time taken for the temperature to rise more than 2 °C being 12 h compared to 32 h for the Lalsil inoculated silage. Bacterial inoculation had no significant effects on the cow's dry matter intake and milk production (Table 1). The protein, fat and urinary nitrogen contents of milk were also not affected by the inoculated silage. However, both milk lactose and solid not fat contents of milk in cows fed inoculated silage were statistically significantly higher (P<0.05) than those in the control group.

Conclusions It can be concluded that low dry matter maize silage inoculated with Lalsil inoculant does not affect daily dry matter intake and milk yield of Holstein dairy cows but does affect lactose and solid-no-fat contents of the milk. Lalsil inoculation may also improve the aerobic stability of maize silage.

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Relationship between starch degradation of cereal grains determined after rumen incubation and by boiling with α -amylase solution

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Introduction Information on starch degradation is required to promote a more efficient use of energy and protein in dairy cow diets. Several *in vitro* and *in situ* methods have been proposed to estimate starch degradability. However, they are not suitable for application in routine analysis as they often require the use of cannulated animals. The objective of this study was to determine starch degradation of cereal grains using a method based on boiling extraction for 30 minutes with α -amylase solution. The starch degradation after incubation *in sacco* for 6 and 24 h was used to evaluate the new method proposed.

Materials and methods Twenty samples of 6 cereal whole-grains (barley, oat, maize, rye, triticale and wheat) were used in this study. Grains (1 mm) were analysed for dry matter (DM) and crude protein (CP) according to AOAC (1990) and neutral detergent fibre (NDF) according to Van Soest *et al.* (1991). Samples ground to pass a 0.5 mm screen were used for starch determination (Solomonson *et al.*, 1984) and for boiling with α -amylase solution. Samples (4 mm) were incubated *in sacco* (Ørskov *et al.*, 1980) for 6 and 24 h. The daily ration of the cannulated rams consisted of maize silage, meadow hay and concentrate making 50:7:43 on DM basis. After incubation, residues were ground (0.5 mm) and analysed for starch as previously described. For the enzymatic method, 500 mg of sample were boiled for 30 minutes with 50 ml α -amylase (Sigma A-3306) solution, in triplicate and repeated twice. After boiling, the content was centrifuged twice at 4000g for 15 minutes and the residues were washed with hot distilled water. Starch was determined on residues as described. The relationship between starch degradation after incubation *in sacco* and after boiling with α -amylase solution was determined using regression analysis.

Results Chemical composition of cereal grains is in good agreement with current tabulated values. The CP ranged from 8.6 (maize) to 12.3% DM (triticale), the NDF from 11.6 (maize) to 31.5% DM (oat) and starch varied from 43.1 (oat) to 76.2% DM (maize). Differences in the starch degradation between cereal grains were found with both methods. Average values of starch degradation estimated after boiling with thermo stable α -amylase solution were higher than values determined after 6h (30.8 vs 63%) of incubation *in sacco* but they are lower than values determined after 24h of incubation *in sacco* (91.7 vs 63%). However, the ranking of cereal grains according to starch degradation was similar with both methods. The higher values were obtained for oat and the lower values were found for maize grains. Correlation coefficient between starch degradation by boiling with α -amylase solution or by incubation *in sacco* in the rumen during 6 and 24h were significant ($r=0.67$, residual standard deviation=2.071% and $r=0.78$, residual standard deviation= 4.122%, respectively; $n=20$; $P<0.001$). Values obtained with α -amylase solution explained 60% of the variation of ruminal starch degradation after 24h of incubation *in sacco*. Figure 1 shows the relationship between starch degradation after boiling with α -amylase solution and after 24h of *in sacco* incubation.

Table 1 Starch degradation (%) of cereal grains.

Grains	<i>In sacco</i> degradation		α -amylase solution 30 minutes
	6h	24h	
Barley (n=2)	32.5	92.0	64.7
Maize (n=10)	25.4	88.4	56.0
Oat (n=2)	34.8	96.5	68.5
Rye (n=2)	30.1	90.8	63.2
Triticale (n=2)	29.0	89.5	60.0
Wheat (n=2)	32.9	92.9	65.6

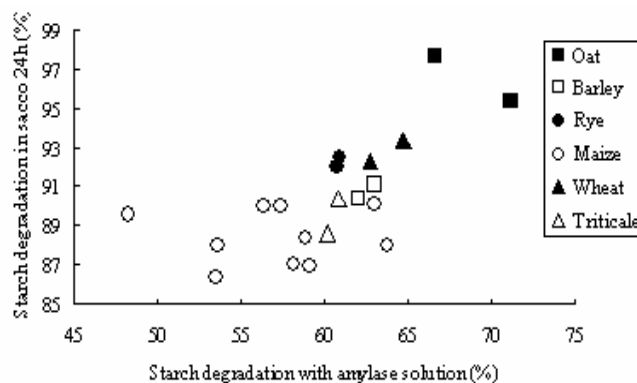


Figure 1 Relationship between starch degradation of cereal grains determined after 24h incubation *in sacco* and by boiling with α -amylase solution.

Conclusions These results show that boiling with α -amylase solution for 30 minutes was able to estimate the starch degradation of cereals grains after 24h of rumen incubation *in sacco*. This approach will further be explored using other *in sacco* incubation times and the degradation constants of the exponential model.

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Nutritional evaluation of low dry matter maize forage ensiled with or without a bacterial inoculant: 1. Effects on fermentation parameters and dry matter degradability

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Introduction Maize silage is the main forage used for dairy cattle in Iran and plays an important role in supplying digestible fibre and energy to these animals. Microbial inoculants are the most commonly used biological additives to accelerate the decline of pH during the initial stage of silage fermentation and to preserve plant carbohydrates through homofermentation which may resulted in an improvement in nutrient digestibility (Aksu *et al.*, 2004). However, their use in silage production has been very limited in Iran. The objectives of this experiment were to study the effects of a bacterial inoculant on the fermentation characteristics and dry matter (DM) degradability of ensiled low DM maize forage.

Materials and methods Maize (*Zea mays* L.) forage was harvested by chopper at a DM of about 220 g/kg for ensiling in 40-tonne (t) capacity silos with or without a bacterial inoculant for two months. The inoculant (Lalsil MSO1, Lallemand, France) contained 10^9 - 10^{10} colony forming units (CFU) per g of each of *Lactobacillus plantarum* (MA18/5U) and *Propionibacterium acidipropionici* (MA26/4U) strains. The inoculant was dissolved in tap water (2.5 g/l) and then sprayed on to the chopped maize forage (1 l/t) at point of ensiling. Four mature Iranian Shal 1×Zandi male sheep (40±3 kg live weight) were used for in sacco degradability studies. The animals were housed individually in metabolism cages with free access to water and mineral blocks. Sheep were fed twice daily at 08:00 and 17:00 h a total mixed ration containing lucerne hay, wheat straw, barley grain, cottonseed meal and wheat bran with forage to concentrate ratio of 75:25 on a DM basis. Silage samples (each about 2 kg) were taken from each silo in triplicates and used for the measurement of fermentation (pH, buffering capacity (BC), ammonia-nitrogen (NH₃-N), water soluble carbohydrates (WSC) and fermentation acids) and *in sacco* DM degradability parameters. Dried samples were milled through a 2 mm sieve and 4 g of each was weighed into nylon bags (45 µm pore size) and were washed (zero time) or incubated in the rumen for 4, 8, 16, 24, 48, 72 and 96 h. After each incubation time, the relevant nylon bags were removed from the rumen and were washed manually and dried for 24 h at 60°C. The DM disappearances at each incubation time was then determined. Degradability data were modelled according to Ørskov and McDonald (1979). Data were analysed by one-way analysis of variance using Minitab to determine significance at P<0.05.

Results The effects of the bacterial inoculate on the fermentation parameters and degradability characteristics of maize silage are presented in Table 1. The pH value and the amounts of NH₃-N and acetic acid were lower and the residual WSC, lactic acid and propionic acid were higher in the inoculated silage compared to the control. Buffering capacity was increased during the ensilage process and it was also higher in the inoculated silage than that of the control. The effective DM degradability was increased in the inoculated silage (p<0.05). The rapidly degradable fractions (a) were higher and the slowly degradable fractions (b) were lower in the inoculated silage but the constant rate of degradation (c) parameters were not differed significantly.

Table 1 Fermentation parameters and degradability characteristics of ensiled maize forages (as g/kg DM unless stated otherwise)

Parameters	Un-ensiled	Ensiled forage		SEM
	forage	Uninoculated	Inoculated	
DM (g/kg fresh)	21.8	20.9	21.3	0.86
pH	6.83	3.75 ^a	3.67 ^b	0.12
BC*	14	91	98.2	3.36
WSC	119.6	14.2 ^a	34.3 ^b	1.73
NH ₃ -N (g/kg total N)	0	0.68 ^a	0.39 ^b	0.023
Lactic acid	4.5	72	77	0.29
Acetic	ND	38.6 ^a	22.5 ^b	1.59
Propionic	ND	6.8 ^a	8.6 ^b	0.24
a (g/kg)	ND	153 ^a	220 ^b	10.6
b (g/kg)	ND	544	455	19.3
c (/h)	ND	0.042	0.045	0.003
P (0.05/h)	ND	410 ^a	464 ^b	6.4

*BC, (meq of NaOH/100 g DM); P, effective DM degradability calculated at given outflow rate; ND, not determined.

Conclusions The results from both the fermentation and degradability measurements indicated that Lalsil inoculation of low DM maize forage affect the fermentation process of forage during ensilage. This may results in the production of better quality silage with lower NH₃-N and higher WSC contents. It was also resulted in the rapidly degradable fractions (a) and the effective DM degradability values of silage to be increased significantly.

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A comparison of the protein degradation profile of soyabean meal and a slow release nitrogen source (Optigen®) *in vitro*

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Introduction There are many rationing models used commercially for evaluating diets fed to dairy cows. A new model – BioParaMilk – uses a unique protein degradation model to determine microbial protein synthesis, based upon the *in vitro* gas production technique (IVGPT). Optigen®, a slow release, blended, non-protein nitrogen source, can partially replace soyabean meal (SBM) in a dairy diet. The partial replacement of soyabean meal and rapeseed meal with Optigen® has been shown to increase fibre digestion and may improve volatile fatty acid (VFA) and microbial nitrogen (N) flow in the rumen (Sinclair *et al.* 2008). The purpose of this study was to evaluate the protein degradation curve from Optigen® compared to SBM for use in this new model, using IVGPT.

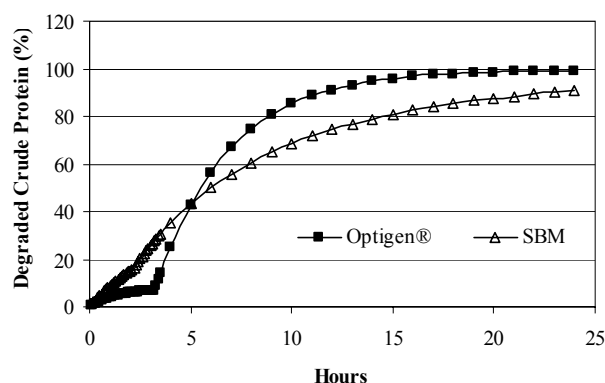
Materials and methods Rumen liquor was collected from a freshly slaughtered sheep and filtered in an attempt to remove all free ammonium. SBM was dried at 60°C for 24 hours and milled through a 2mm grate, whilst the Optigen® was incubated without any preparation. Samples of SBM (545 g crude protein (CP)/kg dry matter (DM)) and Optigen® (2,560 g CP/kg DM) were weighed in triplicate (weights equated to 0.19mgCP/syringe: 27mg and 6mg respectively) into 100 ml glass syringes along with 100mg of sucrose to provide a source of rapidly degradable carbohydrate. The syringes were sealed and 30ml of inoculum (rumen liquor plus nitrogen-free buffer medium (1:2 v/v) was pumped into each syringe. These were placed into a 39°C water bath and the cumulative gas production measured at 30 minute intervals up to 3 hours, then every 1 hour up to 6 hours, every 2 hours up to 12 hours and then every 4 hours up to 48 hours. At 48 hours the entire contents of the syringes were emptied into medicine flat-bottomed bottles, mixed with 15ml of Neutral Detergent Fibre (NDF) solution and autoclaved at 105°C for 1 hour. The contents of the bottles were then filtered through filter paper and the residual nitrogen measured using the Kjeldahl technique. Residual nitrogen values were corrected for blank values and the resulting gas curves fitted to the model: Total Degraded CP (propCP) = (Quick CP (propCP) * (1-exp(-Quick CP rate (-h) * t (h)))) + (Slow CP (propCP) * (1-exp(-Slow CP rate (-h) * (t (h) – lag (h))))).

Results In Table 1 the CP degradation parameters of SBM and Optigen® are shown and the corresponding degradation profiles of these two feeds illustrated in Figure 1. Optigen® was estimated to have a lower level of quickly degraded nitrogen compared to SBM, though a slightly faster rate of degradation in the slow nitrogen fraction. After 15 hours, 95% of the nitrogen contained within the blended non-protein nitrogen product had been degraded compared to 80% in the SBM, with complete disappearance for the non-protein nitrogen (NPN) product after 24 hours. The lag phase was longer for the Optigen®.

Table 1 Protein degradation parameters of soyabean meal (SBM) and Optigen® using IVGPT

	Units	SBM	Optigen®
Crude Protein	g/kg DM	545	2560
Quick Fraction	(prop CP)	0.31	0.08
Quick Fraction Rate	/h	0.334	0.710
Slow Fraction	(prop CP)	0.65	0.92
Slow Fraction Rate	/h	0.114	0.274
Lag time	h	2.14	3.22

Figure 1 Protein degradation versus time of soyabean meal and Optigen®



Conclusions The study confirmed that the nitrogen contained within the blended non-protein nitrogen product (Optigen®) is released at a slow rate and could be used as a partial replacement for soyabean meal. By providing rumen microbes with a steady supply of nitrogen, the use of a controlled release, blended non-protein nitrogen source may improve the efficiency of diet utilisation.

Acknowledgments Financial and product support from Alltech (UK) Ltd is gratefully acknowledged as is the help of Mr Graeme Allen.

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Effect of fresh leaves and residue of oil extraction of eucalyptus (*Eucalyptus citriodora*) on methane emission *in vitro*

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Introduction *Eucalyptus (Eucalyptus citriodora)* is a tall evergreen tree native with many species available in many parts of the world including Australia, China, India, Portugal, Spain, Egypt, Algeria, the southern United States, and South America. There are few experimental data on effects of the eucalyptus fresh and residual leaves material (RLM) from oil extraction on rumen microbial fermentation patterns, therefore the objective of this study was to investigate the effect of eucalyptus fresh and RLM on methane emission and rumen activity *in vitro*.

Materials and methods Eucalyptus fresh and RLM were obtained from the Distillery Tres Barras Company, Torrinha city, Sao Paulo, Brazil at June 2007. The RLM were the by product of the steam distillation of fresh eucalyptus leaves to extract the oil. Lucern (*Medicago sativa*) hay has been used as reference forage. The samples were analyzed according to AOAC (1995) and Van Soest *et al.*, (1991), as well analyzed for extractable total phenols (TP), tannins (TT) and condensed tannins (CT) according to Makkar *et al.*, (1993). The *in vitro* gas production (GP) assay was carried out using a pressure transducer in 160 ml serum bottles incubated for 24h at 39°C and inoculum collected from five sheep according to Bueno *et al.*, (2005). About 0.5 g of each sample was incubated in buffered rumen fluid (2:1, v/v). Three GP runs were done with four replicates for each sample. True dry and organic matter degradability were measured at 24 h. Efficiency of microbial protein *in vitro* was estimated by the mg of truly organic matter degradation: gas volume produced thereby (termed as a partitioning factor, PF (mg/ml) at 24 h incubation) according to Blummel *et al.*, (1997). Protozoa were counted microscopically following the procedure described by Kamra *et al.*, 1991. Means were compared by Tukey test.

Results The crude protein (CP) contents were 76.4, 78.1 and 181.9 g kg⁻¹ DM for eucalyptus fresh and RLM and lucerne, respectively. The neutral-detergent fibre (NDF) and acid-detergent fibre (ADF) were significantly lower in eucalyptus fresh and residue leaves than alfalfa. The Eucalyptus fresh and RLM were rich in TT and TP but had negligible content of CT. There was significant reduction in cumulative gas production about 50% with eucalyptus fresh RLM compared with Lucerne. The methane production (ml/g DM) was reduced by 53 and 55% with eucalyptus fresh RLM, respectively but the reduction was 21 and 16% when expressed on truly digested organic matter basis. This reduction in CH₄ production may be attributed to a decrease in fermentable substrate rather than to a direct effect on methanogenesis. There were significant (P < 0.05) decline in true dry and organic matter degradation *in vitro* in eucalyptus fresh and RLM compared with Lucerne hay substrate. The PF values were significantly (P>0.05) higher in eucalyptus fresh and RLM than Lucerne. There was no significant difference observed between eucalyptus fresh and RLM and Lucerne in protozoa count.

Table 1 The chemical composition (g/kg DM) of eucalyptus fresh and RLM and Lucerne hay.

	CP	NDF	ADF	TP	TT	CT
Lucerne hay	182	547	346	10.2	6.6	0.20
Eucalyptus fresh leaves	76.4	616	504	142	107	4.1
RLM	78.1	604	493	115	104	1.3

Table 2 Effect of fresh eucalyptus, RLM and Lucerne hay on cumulative gas production (GP), methane (ml/g TDOM), partition factor (PF), truly dry and organic matter degradation (TDDM, TDOM g/kg DM) and protozoa count (X10³/ml) incubated with rumen fluid *in vitro* for 24h.

	GP (ml/g DM)	CH ₄ (ml/g DM)	CH ₄ (ml/g TDOM)	PF	TDDM	TDOM	Protozoa
Lucerne hay	111 ^a	11.5 ^a	17.9 ^a	3.9 ^b	670 ^a	645 ^a	4.83 ^a
Eucalyptus leaves	51 ^b	5.4 ^b	14.1 ^b	6.1 ^a	436 ^b	369 ^b	4.85 ^a
RLM	54 ^b	5.1 ^b	15.1 ^b	5.7 ^a	394 ^b	341 ^b	5.15 ^a

a,b means with different subscripts, within column, are significantly differ (P<0.05).

Conclusions This study suggested that that eucalyptus (either fresh or residue) could modify the rumen fermentation and have a potential in methane mitigation, which may be beneficial for improving animal growth.

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The *in vitro* gas production of untreated and high pressure steam treated sugarcane pith

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Introduction Sugarcane pith is a by-product of the final stage of the processing of sugar cane as it passes through rotary sieves to separate fine particle. A technique, which has shown considerable potential as a method for the cost-effective pre-treatment of lignocellulosic material, is steam explosion. At the end of this process, a substantial proportion of the hemicellulose fraction is made water soluble and the lignin fraction is modified. This results in a cellulosic substrate with improved enzymatic accessibility and digestibility. It has been shown that energy availability is increased by solubilisation of cellulose and hemicellulose and/or by freeing digestible materials from lignin or silica (Hart *et al.*, 1981; Horton *et al.*, 1991). By applying the steam explosion process to sugarcane bagasse, Kling *et al.* (1987) demonstrated that about 60% of the hemicellulose fraction was hydrolysed and the susceptibility of cellulose to enzymatic hydrolysis was increased. For treatments based on the use of steam-pressure alone high temperatures are needed ($>180^{\circ}\text{C}$) which may result in the formation of anti-nutritional factors such as 2-furaldehyde (furfural) by secondary dehydration reactions of hemicellulosic pentoses and soluble phenolic compounds. Both of these inhibit the activity of rumen microbes and cell-free enzymes. The objective of this experiment was to evaluate the effect of high-pressure steam treatment on cumulative gas production (GP) profile of sugarcane pith.

Materials and methods Sugarcane pith (2 kg) was either untreated (UTP) or steam treated (STP), at 19 bar for 3 min (70% moisture). Gas production of samples was determined, in triplicate together with three syringes containing only incubation medium (blank) and GP from the sample was corrected for the blank. Using the method of Menke and Steingass (1988) 500 mg air dried, milled sample (1mm screen) was incubated in 100 ml glass syringes with 30 ml of artificial saliva and 15 ml of strained rumen liquor, which was collected from three rumen-fistulated steers (400±40 kg) fed twice daily a diet containing lucerne hay (5.72 kg) plus concentrate mixture (3.08 kg) prior to their morning feeding. Syringes were incubated in 39 °C and GP measured at 3, 6, 12, 24, 48, 72 and 96 h. The cumulative *in vitro* GP data was fitted to the exponential equation $Y = b(1 - e^{-ct})$. Total soluble sugars (TSS) content of STP and UTP was measured by the phenol-sulphuric method (Dubois *et al.*, 1956). Data were analysed by GLM procedure with used the effect of steam as treatment in complete randomised designed (SAS, 2002).

Results The TSS content and cumulative *in vitro* GP of the UTP and STP is given in Table 1. High-pressure steam treatment substantially increased the TSS of sugarcane pith. Using high-pressure insignificantly increased the GP (7%), also there was no difference between rate of GP (c) between treated and untreated pith ($P>0.05$).

Table 1 Total soluble sugar content and cumulative *in vitro* gas production (ml per 500 mg dry mater) of untreated (UTP) and high-pressure steam treated (STP) sugarcane pith (Mean± SE)

Item	UTP	STP	s.e.m	Effect
TSS, mg/g DM	20.3	123.8	15.2	*
GP 24h	37.30	40.30	1.62	NS
b	110.6±2.7	122.5±6	6.9	NS
c	0.018±0.0	0.015±0.001	0.002	NS

b= GP from fermentable fraction NS= non significant difference c= rate constant of GP
s.e.m= standard error of mean * $P<0.05$

Conclusions The results of the present study indicated that use of high-pressure steam for treatment of sugarcane pith solubilised the hemicellulose fraction which resulted in a substantial increased in TSS. Although TSS increased there was no effect of treatment on total GP. Liu *et al.* (1999) demonstrated the inhibitory effect on the GP at the higher steam pressures may be attributed to some anti-nutritional compounds produced during steam treatment. Therefore, further work is required to determine the effect of steam treatment of sugarcane pith on animal performance and their rumen micro-organisms.

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Degradability of pistachio by-products at different harvesting times

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Introduction Iran is a largest pistachio producer in the world. Typically about 300,000 tonnes were exported to other countries per annum. Total pistachio by-product production in Iran has increased at an average rate of about 400,000 tonnes per year, and is becoming an environmental problem. Pistachio hulls are produced during de-hulling of pistachio nuts soon after harvest. Pistachio hulls contain about 12% crude protein (CP), 5% ether extract, 34% NDF, 21% ADF, 9.20% ash and 8% tannin (DM basis). Using pistachio by-products for ruminant feed could reduce the feed cost, environmental pollution and associated difficulties. This study was conducted to determine whether there is a variation in nutritive value of pistachio by-product during harvesting time.

Materials and methods Pistachio was harvested throughout August 2006. Pistachio by-product samples were taken from three pistachio processing factories during early, mid- and late August. For each harvesting time, three samples from the three factories were pooled, mixed and sub-samples taken, dried and ground to pass a 2mm screen. Five grams of dry matter (DM) were weighed into nylon bags (10mm×20mm; with pore size $51\pm 2\mu\text{m}$) for *in situ* degradability measurement using 3 fistulated steers fed a 40:60 concentrate: forage diet. For each time point (0, 2, 4, 8, 16, 24, and 48h), three nylon bags were placed in the rumen. After removal from the rumen, the bags were washed with tap water until the rinse water was clear, and placed in an oven at 55°C prior to weighing. Kinetics of DM disappearance *in situ* was estimated using the nonlinear regression procedure of SAS (2002). The following model was used to the percentage of disappearance of DM: The DM degradability coefficient was determined using the equation, $P=a+b(1-e^{-ct})$. Effective ruminal degradability percentage of DM (EDDM) was calculated by the equation $P=a+bc/(c+k)$. Data were analysed by analysis of variance and differences between means were tested with Duncan's test.

Results The DM degradability of the pistachio by-products is shown in Table 1. The second harvesting time (11 August, 2006) had a lower degradable fraction (a and b) compared with other harvesting times ($P<0.05$). There were significant differences among the potential degradable fraction (a+b) of pistachio by-products ($P<0.05$) at different harvesting times. The EDDM values did not differ significantly between by-products. The effect of different harvesting time (maturity stage) on DM degradability is shown in Figure 1.

Table 1 Degradable coefficients of DM of pistachio by product (different harvesting time)

	a (%)	b (%)	c (%)	a+b (%)	EDDM (%)
1 August, 2006	48.37 ^a	39.14 ^a	0.073	87.48 ^a	71.89
11 August, 2006	39.86 ^b	35.48 ^b	0.066	85.35 ^b	70.35
21 August, 2006	47.01 ^{ab}	38.12 ^a	0.063	85.14 ^b	70.04
SE	0.41	0.23	0.53	0.65	0.73

a: rapidly degradable fraction, b: slowly degradable fraction, c: fractional degradation rate constant (%/h).

Conclusion In general, the results of this experiment suggest that DM disappearance of pistachio by-products decrease as harvesting times progress. The reason for this can be contributed to maturity of pistachio by-products such as leaves and other parts of plants. This reduction in DM degradability may be because of an increase in NDF and tannin content of pistachio hulls.

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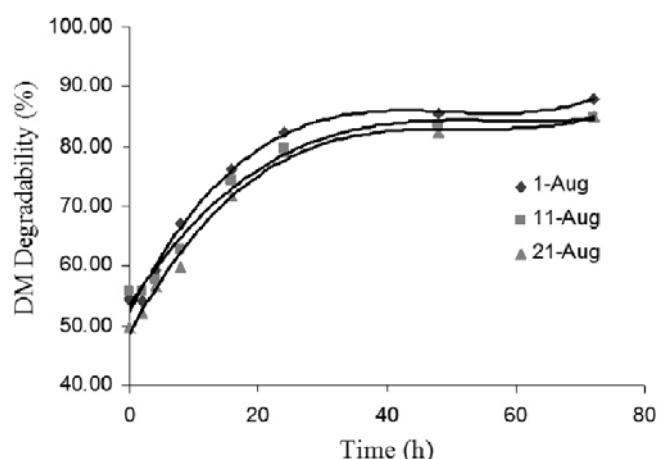


Figure 1 Effect of harvesting time on DM degradability

Effect of dietary protein concentration on performance of group-fed pigs

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Introduction Dietary protein is the main source of nitrous oxide and nitrates, harmful pollutants which are produced in pig units. So reducing the level of protein in the diet may be environmentally friendly. But will this compromise performance? This study compared three ‘nutritional strategies’ providing different protein and energy levels as pigs of two breeds grew from 40 to 120kg live weight.

Materials and methods One hundred and ninety-two entire male pigs were used, half 75% Large White (LW) and half 75% Duroc (D). They were reared on one of three ‘nutritional strategies’ from 40 to 120kg live weight at Harper Adams as shown in Table 1.

Table 1 Nutritional strategies imposed on group-fed Large White and Duroc pigs

Wt range	‘Baseline’ (B)			‘State of the art’ (SOTA)			‘Low Protein’ (L P)		
	DE ^a	CP ^b	L ^c	DE	CP	L	DE	CP	L
40-65kg	13.5	210	12	14.0	195	12	14.0	195	12
65-90kg	13.5	210	12	13.5	180	11	13.5	165	10
90-120kg	13.5	210	12	13.0	170	10	13.0	130	7

^aMJ Digestible Energy/kg ; ^bg/kg crude protein; ^cg/kg lysine

The dietary ingredients were the same in all strategies (barley, wheat, wheat feed, rapeseed meal, soyabean meal) and ‘State of the art’ (SOTA) and ‘Low protein’ (LP) diets were balanced with amino acids so the ratios with lysine were the same as in ‘Baseline’ (B) diet. The pigs were fed *ad libitum* from hoppers and reared in groups of 8 pigs per breed x nutritional strategy. There were 4 replicates of the experiment. At approximately 120kg live weight, the pigs were transported to the University of Bristol abattoir where they were slaughtered and samples were removed for analysis. For individual pigs (n=32 per breed x nutritional strategy), slaughter weight, carcass weight, P₂ fat thickness (skin plus subcutaneous fat measured 6.5 cm from dorsal midline at level of last rib) and daily liveweight gain were statistically analysed using general linear models with nutritional strategy and breed as factors; for pens (n=4), intakes and food conversion ratios (FCR) were similarly analysed.

Results The mean values for growth parameters over the period 40 to 120 kg and P₂ fat thickness of the pigs at 120kg, are shown in Table 2.

Table 2 Growth, feed intake, nutrient utilisation and fat thickness in Large White and Duroc pigs

Breed	Large White			Duroc			sed	prob.
	Strategy	B	SOTA	LP	B	SOTA		
Sl wt(kg)	123.6	121.5	124.5	121.2	122.4	123.1	1.673	0.378
Carc wt (kg)	91.02	89.32	92.50	90.20	90.96	92.00	1.298	0.333
P ₂ (mm)	13.80	15.00	18.41	17.94	18.86	19.03	0.858	0.006
DLWG ^a (g)	1030	1024	999	1003	1010	1011	24.24	0.516
Intake/d (g)	2911	2732	2800	3061	2998	2880	121.2	0.562
FCR	2.85	2.67	2.87	3.09	2.98	2.89	0.105	0.173
DE ^b /kg gain	38.34	36.95	38.42	41.73	39.90	38.79	1.457	0.208
N ^c /kg gain	96.93	76.40	71.02	103.4	84.13	71.73	3.395	0.323

^aDaily liveweight gain; ^bMJ; ^cg

Of the data recorded on individual pigs, only P₂ fat thickness was significantly different between factors, being greater in D than LW and in LP than B and SOTA. The small numbers of observations for data recorded on a pen basis was partly responsible for the lack of significant effects for intake parameters. When breeds were pooled, there were no significant effects of nutritional strategy on these parameters except for N intake/kg gain which was lowest in LP (Table3).

Table 3 N intake/kg gain in pigs subjected to the three nutritional strategies (breeds pooled)

Strategy	B	SOTA	LP	sed	Prob.
N/kg gain (g)	100.17	80.27	71.38	2.401	P<0.001

Conclusions These results show that reducing protein to a low level (120g/kg) in pig diets in the later stages of growth reduced N intake, a major factor in N losses to the environment, with relatively few deleterious effects on performance except that fat thickness was increased, especially in the leaner LW breed. This suggests that energetic efficiency was increased on the low protein strategy.

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Effect of a low protein : energy nutritional strategy on intramuscular and subcutaneous fatty acid content, and stearoyl-CoA desaturase protein expression in Large White and Duroc pigs

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Introduction The production of pigs with low subcutaneous but high intramuscular fat (IMF) is one of the main challenges of the pig industry, and it requires the understanding of the mechanisms controlling fat formation in these two depots. IMF can be increased by reducing the ratio of protein to energy in pig diets (Wood *et al.*, 2004). This is accompanied by activation of expression of the enzyme stearoyl-CoA-desaturase (SCD), which catalyses the biosynthesis of monounsaturated fatty acids (MUFA) (Doran *et al.*, 2006). It is well known that IMF content varies between breeds. However it remains unclear as to whether pigs with predominantly high IMF (e.g. Duroc) will respond to a low protein diet in the same way as those with predominantly low IMF (e.g. Large White). The objective of the present study was to investigate whether the effects of low protein: energy diets on IMF, MUFA and total fatty acid content are breed-specific.

Material and methods Twenty-eight pigs (entire males) with either 75% Large White (LW) or 75% Duroc (D) genes were used. Within each breed half were fed the same levels of crude protein (CP)/digestible energy (DE) in three growth stages (40-65, 65-90 and 90-120kg liveweight), i.e. 21.0% CP, 13.5 MJDE and 1.2% lysine. This control regime (C) was contrasted with a low protein (LP) regime in which DE and CP were respectively: 40-65kg pigs: 14.0, 19.5; 65-90kg pigs: 13.5, 16.5; 90-120kg pigs: 13.0, 13.0. Fatty acid (FA) composition of *longissimus dorsi* muscle (LD) and subcutaneous adipose tissue was analysed by high resolution gas chromatography. SCD expression was analysed by the Western blotting technique. Statistical significance of differences between means was estimated by the Student's t-test.

Results The LP regime significantly increased SCD protein expression in muscle of LW but not D pigs (Table 1). In contrast to muscle, there was no significant effect of the LP diet on SCD expression in subcutaneous fat. In LW pigs all MUFA concentrations and total FA (marbling fat) tended to increase on the LP regime. The effect of LP on MUFA concentrations in D pigs was lower than in LW.

Table 1 Effect of a low protein regime on total fatty acid (FA) and MUFA contents (mg/100g of tissue), and SCD protein expression (arbitrary units) in LD muscle and subcutaneous fat of Large White and Duroc pigs

Parameter	Large White				Duroc			
	C n=7	LP n=7	t	(df, P)	C n=7	LP n=8	t	(df, P)
Muscle								
C16:1	28.5	43.4	1.36	(12, 0.198)	83.5	79.1	0.24	(13, 0.812)
C18:1n-9	278.7	457.3	1.50	(12, 0.159)	910.2	852.7	0.26	(12, 0.801)
Sum MUFA ¹	357.0	573.7	1.48	(12, 0.164)	1129.6	1053.0	0.28	(12, 0.781)
Total FA	980.6	1378.8	1.43	(12, 0.178)	2546.3	2413.7	0.24	(12, 0.815)
SCD	61.1	83.4	3.48	(13, 0.004)	68.5	75.9	0.62	(13, 0.544)
Subcutaneous fat								
C16:1	1845.1	1992.0	1.57	(7, 0.161)	1603.7	1684.2	0.89	(13, 0.391)
C18:1n-9	23370.7	26304.2	2.44	(12, 0.031)	23544.6	23780.2	0.23	(13, 0.821)
Sum MUFA	28552.9	31902.9	2.51	(12, 0.027)	28408.0	28595.9	0.16	(13, 0.875)
Total FA	69524.5	74684.9	1.75	(12, 0.105)	73185.9	73107.7	0.03	(9, 0.973)
SCD	102.5	117.1	1.80	(12, 0.098)	78.7	85.3	0.37	(12, 0.717)

¹Sum MUFA was calculated as sum of C16:1, tC18:1n-7, C18:1n-9, C18:1n-7 and C 20:1 fatty acids

Conclusions This study demonstrates that the response of pigs to a low protein:energy regime is breed-specific. The response of LW pigs (in terms of individual and total MUFA content and SCD protein expression) was higher in comparison with D pigs. The results contribute to understanding the mechanisms controlling IMF formation.

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A model on biological flow of phosphorus in growing pigs

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Introduction Phosphorus is the second most plentiful mineral in the animal body. It is present as structural component and it is involved in a wide variety of biochemical reactions. Research has been conducted in Brazil to study phosphorus metabolism in pigs using the model of Fernandez modified by Lopes *et al.* (2001). However there is little published information related to phosphorus metabolism in pigs using the model of Vitti *et al.* (2000). In the present study, it was considered the hypothesis that P intake regulates P utilisation and flow on gut, blood, soft tissues and bone. The isotopic technique by using labelled P allows the formulation of model of the biological flow of phosphorus in the animal organism. The aim of this study is to evaluate the effects of phosphorus intake on phosphorus metabolism in pigs, by using isotope and balance techniques. A mathematical model for phosphorus metabolism is formulated.

Materials and methods The research was based on model of Vitti *et al.* (2000). The data were analyzed in a completely randomized design. Twenty crossbred barrows, with means weight of 20 kgLW, were housed in metabolic cages designed for studies and handling of faeces and urine, for a seven day period for adaptation and other seven days to obtain the total collection of faeces, urine and blood samples. The pigs received diets consisting of different phosphorus levels: treatment A- diet based on corn, soybean meal and vitamin and mineral supplementation (except phosphorus), with 0.30% of total phosphorus. The adjustment of the total phosphorus in the other treatments was obtained by addition of dicalcium phosphate by giving the following total P levels: treatment B- 0.40%; C - 0.51%; D- 0.65% and E- 0.73%. Phosphorus levels in the experimental diet were deficient for treatments A and B, adequate for treatment C and in excess for treatments D and E.. Feed was given twice a day.

Results The results from the present experiment are summarized at Tables 1 and 2. The increased P excretion in urine in response to P ingestion shows that in non- ruminant animals the kidneys are an important route for regulating P homeostasis, beside P excretion in faeces. In the present study, despite the differences in P intake, it was not verified effect in bone re-sorption. This could be due to the short experimental period (14 days) and the healthy conditions of the animals at the beginning of the experiment. When P re-sorption is compared to the total P absorbed (F_{23}/F_{21}), it is observed that for low P treatments that higher amount of P is mobilized from bone to the central pool. This seems a mechanism to maintain P levels in plasma. The increased output of P from soft tissues for low P treatment also contributes to maintain P levels in the central pool for the normal functions of the animal body. The faecal and urinary losses of phosphorus regulate the P homeostasis in growing pigs.

Table 1 Phosphorus intake levels and phosphorus excretion in pig

Input (g of P/d)	Level of phosphorus intake				
	A	B	C	D	E
Intake	^d 1.88	^c 2.92	^b 3.8	^b 4.55	^a 5.63
Faeces	^b 1.25	^{ab} 1.52	^b 1.3	^{ab} 1.88	^a 2.35
Urine	^{ab} 0.05	^b 0.09	^{ab} 0.1	^{ab} 0.33	^a 0.53

^{a,b,c,d} Subcolumn means within row treatment category with different superscripts differ ($P < 0.05$).

Table 2 Comparison of kinetic model outputs for the different phosphorus intake in pigs

Model output (g/d)	Level of phosphorus intake				
P blood to gut	^c 0.26	^{bc} 0.3	^a 0.87	^{abc} 0.55	^{ab} 0.61
P gut to blood	^c 0.89	^b 1.7	^a 3.36	^a 3.23	^a 3.89
P blood to bone	^a 5.23	^a 6.7	^a 8.14	^a 8.24	^a 7.12
P blood to soft	^b 2.40	^{ab} 3.4	^a 4.70	^{ab} 3.53	^{ab} 3.36
P bone to blood	^b 1.92	^a 3.4	^{ab} 2.8	^{ab} 2.30	^{ab} 2.23
P soft tissue to	^a 5.13	^a 5.3	^a 7.70	^a 7.12	^a 5.50

^{a,b,c} Subcolumn means within row treatment category with different superscripts differ ($P < 0.05$).

Conclusions The interchanges of phosphorus from digestive tract and soft tissues to a central pool are related to P intake. Phosphorus intake has no influence on phosphorus mobilization in bone in growing pigs. The model formulated based on the use of radioisotopes is coherent with the biological flow of phosphorus in pigs.

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Kinetics of phosphorus in pigs fed diets with increasing levels of phytase

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Introduction Non-ruminant animals do not synthesize phytase enzyme, a substance capable to act on phytate molecule to liberate phosphorus. The non-available phosphorus pass through the digestive system of the non-ruminants practically without being digested and the inclusion of inorganic phosphorus sources in diets of those animals is necessary. The use of phytase enzyme in rations for pigs can contribute to the elimination or reduction of the use of inorganic phosphorus sources and reducing the environmental impact caused by the excretion of this mineral. The experiment was designed to evaluate the biological phosphorus flow in pigs fed with diet containing different phytase levels, using the isotope dilution technique.

Material and methods Twelve crossbred pigs, barrow males, 31.68±1.62 kgLW, were used. The experimental treatments consisted of phytase levels (253, 759, 1265 and 1748 phytase units/kg of diet) added to a basal diet containing corn, soybean meal and 17.5% of nonfat rice bran. The study was based on model of (Vitti *et al.* 2000). At the first experimental day, 7.4 MBq of ³²P (Na₂HPO₄) was intravenously injected in each animal and blood, faeces and urine samples collected for 7 days. On the 7th day the animals were slaughtered and samples of tissues were collected. The data were analyzed in a completely randomized design.

Results Effect of phytase enzyme on different parameter are shown in Tables 1 and 2. Phytase levels affected P absorption and bone retention (P<0.05). The best response was observed for lowest phytase levels.

Table 1 Effect of phytase enzyme on intake and excretion

	Phytase enzyme (PU/Kg diet)			
	253	759	1265	1748
Input (g of P/d)	253	759	1265	1748
Intake	10.05	9.93	8.87	9.34
Faeces	1.62	1.65	1.59	1.55
Urine	0.020	0.012	0.010	0.018

^a Linear effect (P<0.05).

Table 2 Effect of phytase enzyme on P flow

	Phytase enzyme(PU/kg diet)			
	253	759	1265	1748
Model outputs, g/d	253	759	1265	1748
P from blood to gut	18.31	6.04	7.31	24.28
P from gut to blood	22.81	9.82	10.05	27.64
P from blood to bone	17.11	10.14	10.94	8.90
P from blood to soft tissue	2.88	1.41	2.94	4.45
P from bone to blood	13.19	7.00	9.70	7.58
P from soft tissue to blood	2.31	0.80	1.46	2.73
^a True absorption	4.47	3.77	2.73	3.35
^a P retention bone	3.91	3.15	1.25	1.33

^a Linear effect (P<0.05).

Conclusions The use of isotope dilution technique was useful to study P flows in pigs. The proposed model was efficient for interpretation of the data in the present study and it illustrates the effects of the phytase in the metabolism of P in pigs.

Acknowledgements This experiment is part of projects supported by FAPESP (06/57574-5; 04/14532-5).

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Digestive physiology of pigs fed diets containing different phosphorus sources

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Introduction The metabolism and kinetics model of Fernandez modified by Lopes *et al.* (2001) is a fundamental tool for the study of the digestive physiology of phosphorus (P) in pigs. In Brazil there is a great potential of use of rock phosphate and those represents approximately 2.9 billion metric tons. Brazilian researchers are interested to evaluate those phosphate as alternative sources in diets of pigs. All of the natural sources of phosphate in Brazil presented low level of fluor, when compared with international sources and levels of heavy metals are considered low too. The main concern resides in the fact that non-absorbed P is excreted and can cause contamination of water. The purpose of this research was to evaluate the digestive physiology of pigs fed diets supplemented with different P sources and the study the environmental pollution provoked by P excreted.

Material and methods Twenty-one male pigs averaging 34.94± 2.88 kgLW were fed with diets containing corn and soybean meal supplemented with different P sources: diet control without supplemental P (Cont), dicalcium phosphate (FBC), monocalcium (MBC), triple superphosphate (ST), simple superphosphate (SS), Catalão rock (Rocha) and phosphoric acid (AcF). The animals were kept in metabolic cages and the experimental design used was in randomized blocks. A compartmental model was used to evaluate the digestive physiology of pigs. The parameters evaluated were: P intake (V_i), Fecal P from dietary origin (V_{FD}), Relationship V_{FD}/V_i , absorbed dietary P V_{aa} , bioavailability and P balance in the bones.

Table 1 Digestive physiology of pigs fed with different P sources

Parameters	Sources of phosphorus							
	CONT	FBC	MBC	ST	SS	AcF	ROCHA	EPM
P intake (V_i) *	123.44 ^b	224.63 ^a	221.90 ^a	223.34 ^a	227.27 ^a	223.07 ^a	222.96 ^a	7.97
Fecal P of from dietary origin (V_{FD})	61.06	91.80	87.29	90.66	82.78	83.46	111.41	23.61
Relationship V_{FD}/V_i	0.50	0.41	0.39	0.41	0.36	0.38	0.50	0.11
Absorbed dietary P V_{aa} *	62.37 ^b	132.83 ^b	134.60 ^b	132.69 ^b	144.43 ^a	139.61 ^a	111.55 ^{ab}	26.66
Bioavailability	49.35	58.81	60.81	59.56	63.99	62.37	49.92	12.03
P balance in the bones*	43.16 ^a	87.60 ^{ab}	101.42 ^b	100.28 ^b	108.31 ^b	95.63 ^b	86.52 ^{ab}	19.80

*Means followed in the same line with the same letter don't differ by test.

Results The result of the parameters evaluated are summarized in Table 1. It was observed statistic effect in the variables P intake, absorbed dietary P (V_{aa}), bioavailability and P balance in the bones ($P < 0.05$). Among the P sources studied the simple superphosphate presented the best relationship Intake P/excretion P in feces (1g/ 0.36), 12% lower than dicalcium phosphate. The animals fed Catalão rock presented the relationship P intake/excretion P (1.0 g /0.50) 28% greater than animals fed simple superphosphate (SS).

Conclusions The model developed in this study could be used to simulate the biological flow of phosphorus in pig metabolism. The different phosphorus sources (dicalcium (FBC), monocalcium (MBC), triple superphosphate (ST) and simple superphosphate (SS), could be used in diets for growing pigs. The simple superphosphate (SS) present best results.

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The effect of different levels of palm olein oil on performance trait, egg quality, egg cholesterol and immune system of layers

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Introduction The palm grows well in wet, humid parts of tropical Asia (mainly South-east Asia), Africa, and Central and South America Palm oil is a greatest oils the entire world. The palm oil has a greatest saturated fatty acid. The main aim of this study was to determine the best level of palm olein oil on production performance (gain body weight, egg production, egg weight, egg mass, FCR and feed intake), egg quality (Haugh unit, yolk index, yolk colour index, shell thickness and specific gravity), egg cholesterol and blood factors of laying hens of Leghorn W36.

Materials and methods Eighty four 26-wk-old white leghorn laying hens were randomly assigned to 4 treatment diets, with 3 replicates and 8 layers in each replicate. The experiment was conducted over a period of 12 wk. The diets were isocaloric and iso-nitrogenous and contained 0, 1.5, 3, and 4.5% of Palm olein Oil. Egg quality, egg production, egg weight, body weight and feed consumption were recorded and feed conversion ratio (FCR) was calculated during the experiment. At the end of experiment period, 2 layers per replicate were randomly selected, weighted, and bled, after the blood sampling, antibody against (Newcastle disease) ND and (infectious bursal disease) IBD titer and blood cholesterol determined the end of period.

Results The results of analysis of variance for gain body weight, feed consumption, FCR, blood cholesterol are presented in Table 1 and for egg quality; egg production and yolk cholesterol are shown in Table 2.

Table 1 Results of pair comparisons * among treatments ** for gain body weight, feed intake and FCR

Performance	Treatments				SEM
	T ₁	T ₂	T ₃	T ₄	
Gain body weight	90.0 ^b	98.0 ^a	118.0 ^{ab}	170.0 ^a	19.5
Feed intake/day	93.51	95.51	92.41	94.74	1.51
FCR	1.77	1.82	1.87	1.86	0.033
Blood cholesterol(mg/dlit)	143.00 ^b	157.67 ^{ab}	161.00 ^a	162.33 ^a	9.96
IBD titer	6812	6075	5590	5520	742.78
ND titer	8.67	8.33	8.33	8.67	0.67

- Treatments with different letters were statistically significant (p<0.05) from each other.

**T₁: diet containing 0% Palm olein oil, T₂: diet containing 1.5% Palm olein oil, T₃: diet containing 3% Palm olein oil, T₄: diet containing 4.5% Palm olein oil.

Table 2 Results of pair comparisons among treatments for performance, egg quality, cholesterol and ND titre

Carcass yield and proportions	Treatments				SEM
	T ₁	T ₂	T ₃	T ₄	
Egg production	90.24	91.67	90.46	89.97	1.18
Egg weight	57.73	57.70	56.19	55.91	0.472
Egg mass	52.02	51.76	51.65	49.61	0.808
Egg shape	76.69	76.11	76.65	77.23	0.618
Yolk index	41.83	40.23	42.23	41.43	0.722
Yolk colour index	5.50	5.45	5.56	5.91	0.127
Haugh unit	87.18	85.81	89.08	87.04	0.66
Yolk cholesterol(mg/g)	12.55	13.55	13.65	13.87	0.785
Specific gravity	1.0843	1.0833	1.0846	1.0822	0.0009

T₁: diet containing 0% Palm olein oil, T₂: diet containing 10% Palm olein oil, T₃: diet containing 20% Palm olein oil, T₄: diet containing 30% Palm olein oil

Conclusion The result obtained in the present study showed that increasing different levels of Palm olein oil increased the gain body weight and blood cholesterol from the 90 and 143 (T₁) to 170 and 162.33 (T₄) during the experimental period (P<0.05). The treatments applied had no statistical significant effect (P>0.05) on feed intake, egg quality, yolk cholesterol, IBD and ND titer. Therefore, it is concluded that Palm olein oil at 3 percentage (T₃) of the ration was the best treatment among the other treatments considered in the present research.

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The effect of probiotic supplementation on egg quality of old broiler breeder hens

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Introduction The egg shell quality is related with bird age and is an important factor to poultry industry due to reproduction and economic implications (Peebs and Brake, 1987). Probiotics are biostimulators and immunomodulators containing live or lyophilized bacterial cultures, which regulate and optimize the rations among the different types of microorganisms in the digestive system, preventing upsets and exerting a stimulating effect on the disintegration and absorption of the nutrient substances (Nickolova and Penkov., 2004). Its stimulating effect on nutrients absorption possibility can improve the mineral availability such calcium and also egg shell quality. In some cases positive effects of probiotic (on damaged egg ratio; Balevi., 2001) or lactobacillus (on haugh units; Nahashon *et al.*, 1994b) supplementations have indicated in layer hens. So possibility they could have positive effects on egg quality. Therefore this research was undertaken to assess the effects of probiotic (protexin) on egg quality (thickness, egg shell percentage, strength egg shell, egg shell weight and haugh unit) of old broiler breeder hens.

Materials and methods One hundred sixty 64-week-old broiler breeder hens (Hubbard classic) were involved in this experiment for 10 week. The animals were randomly divided into 16 groups and were randomly housed in 16 different pens. The basal diet was supplemented with a commercial probiotic (protexin® probiotic International Limited, Uk) at levels of 0, 0.25%, 0.5% and 0.75%. The four treatments (A; 0%, B; 0.25%, C; 0.5% and D; 0.75%) were assigned as completely randomized design. Egg shell thickness was determined using a mitutoyo caliper 7313 micrometer with 0.01 mm (0.01- 10 mm) precision. Egg mass and egg quality characteristics (egg shell thickness, egg shell weight, egg shell strength, haugh unit) in each replicate measured during the experimental period. Albumen height measured using a micrometer and haugh units calculated using the formula described by Roush (1981). Differences among experimental treatments were tested by analysis of variance using the General Linear Model (GLM) procedures of Statistical Analyses System and considered significant at a probability $p \leq 0.05$. Differences between treatments were tested using Duncan's multiple range tests.

Results The results of egg mass and egg quality characteristic are shown in Table 1. As seen in this Table, there were no significant differences in egg mass and egg quality characteristics (percentage of egg shell, shell thickness, egg specific gravity, and egg shell strength and haugh unit) among treatments with respect to whole period of the experiment.

Table 1 The effect of probiotic supplementation (protexin®) on egg mass and egg quality characteristics

Variables	Treatments ^a				SE
	A	B	C	D	
Egg specific gravity	1.09	1.08	1.09	1.08	<0.01
Egg shell percentage	9.26	9.13	9.29	9.21	0.09
Egg shell strength (kg/cm ²)	2.26	2.08	2.25	2.16	0.05
Egg shell thickness (mm)	0.32	0.31	0.32	0.31	<0.01
Haugh unit	84.86	85.57	83.4	85.64	0.66
Egg mass (gr)	38.96	40.09	39.81	39.68	0.75

^aTreatments are: A (unsupplemented), B (supplemented with 0.25% probiotic), C (supplemented with 0.25% probiotic), D (supplemented with 0.25% probiotic)

Conclusions Results of our study shows that probiotic supplementation don't have any significant effect on the mentioned variables of eggs in old broiler breeder hens. Several author's (Timms., 1968 and Naqi *et al.*, 1984) cited that the lack of effect of lactobacillus on hen-day egg production and feed conversion ratio may be explained by lactobacilli become established ideal the gut of most species of animal soon after birth. Here, in our work probiotic supplementation was done on the older (after the 64-week of age) broiler breeders and this reason possibility has affected our results.

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Effect of feeding periods and sodium levels of pre-starter diet on broiler performance and serum electrolytes

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Introduction In the last ten years, interest in early nutrition research has increased due to the high correlation between 7-d-old weight and final weight. Sodium (Na), chlorine (Cl) and potassium (K), are essential elements in chickens in order to maintain the osmotic pressure and acid-base balance within normal values, protein synthesis in tissues, maintain extra and intracellular homeostasis and the electric potential of the cell membrane. Electrolyte balance is defined as (Na+K-Cl mEq/Kg). Many studies have indicated that sodium supplementation in the first week of life stimulated feed intake and modified weight gain and feed conversion ratio. The purpose of this trial was to evaluate the effects of different levels of sodium and electrolyte balance in pre-starter diets in three periods of 4, 7 and 10 days on performance and serum electrolytes levels in broiler chickens.

Materials and methods 240 day-old Ross (308) male broiler chickens were used in this trial. In a completely randomized design, chickens were divided into nine treatments of three replicates each according to a factorial arrangement of three periods of feeding pre-starter diet (4, 7 and 10 days) and three dietary sodium levels (0.15, 0.30 and 0.45%), in the pre-starter diets. The electrolyte balances were 200, 250 and 300 meq kg⁻¹. The experiment was conducted in batteries with eight birds per cage, and a total of 27 experimental units. In addition to these treatments, there was a control diet (starter diet with 0.20% sodium) with 200 meq kg⁻¹ diet. The calculated electrolyte balance was obtained by addition of the NaCl, NaHCO₃ and NH₄Cl contents. Chickens were fed starter diet to 21 days of age after feeding pre-starter diet. Grower diet was fed from 22-42 days of age. At 42 days of age, blood samples were collected from two chickens from each experimental unit. Serum electrolytes (sodium, potassium and chloride) levels were measured by flame spectrophotometry and routine biochemical methods (AOAC, 1990). Data were analyzed based on the general linear models procedures of SAS (1998). Significant differences among treatment means were analyzed by Duncan multiple range test at P < 0.05.

Results In the starter period, chickens fed pre-starter diet for seven and ten days ate more feed and had more weight gain than those of chickens fed the pre-starter diet for only four days (P<0.05). Pre-starter diet with 0.30% sodium and 250 mEq kg⁻¹ electrolyte balance had greater weight gain for the first three weeks and feed intake for the first and second weeks of the study (P<0.05). Different periods of feeding pre-starter diet and different levels of sodium and dietary electrolyte balance had no significant effect on feed conversion ratio (FCR) and serum electrolyte level, but interaction among these factors showed chickens fed pre-starter diet with 0.30% sodium for ten days had the lowest FCR in their second and fourth weeks of age (P<0.05). Weight gain and feed conversion ratio in chickens fed pre-starter diet for the first seven and ten days of life, were significantly more than that of the control diet (P<0.05).

Table 1 Effect of periods of feeding pre-starter diet and sodium levels on feed intake and weight gain at starter period

Treatment	Starter period	
	Feed intake (g)	Weight gain (g)
4 day	881.51 ^b	533.30 ^b
7 day	946.11 ^a	575.23 ^a
10 day	970.38 ^a	594.38 ^a
± SE	14.85	11.65
	Sodium level in diet (%)	
0.15 ¹	908.59	570.38 ^{ab}
0.30 ²	957.85	595.22 ^a
0.45 ³	924.34	536.77 ^b
± SE	14.85	11.65

Note: values in each column with different superscripts are significantly different (P<0.05).

¹200 mEq/kg electrolyte balance.

²250 mEq/kg electrolyte balance.

³300 mEq/kg electrolyte balance.

Table 2 Comparison of treatments with control diet

Treatment	Weight gain (g)	FCR
Control treatment	1605.08	2.42
4 days, 0.15% sodium	1739.31	2.30
4 days, 0.30% sodium	1799.29	2.20
4 days, 0.45% sodium	1784.96 *	2.23 *
7 days, 0.15% sodium	1895.98 *	2.06 *
7 days, 0.30% sodium	1856.38 *	2.20 *
7 days, 0.45% sodium	1784.04 *	2.27
10 days, 0.15% sodium	1875.48 *	2.13 *
10 days, 0.30% sodium	1912.86 *	2.13 *
10 days, 0.45% sodium	1777.19 *	2.42 *

Note: values in each column with * superscripts are significantly different (P<0.05).

Conclusions It was concluded that body weight gain and FCR in chickens fed pre-starter diet for the first seven and ten days of life with 0.3% sodium was improved and this practice could be recommended to broiler producers.

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Interactive effect of sodium bentonite with pigment on performance and egg quality of laying hens from 36-48 weeks of age

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Introduction Sodium bentonite (SB) has been widely used in poultry industry as agent that binds mycotoxine, moisture and ammonia. SB supplementation has generally been used in animal diets for reasons other than the nutrients they supply. 3% SB in cattle rations reported no significant influence on rate of gain, feed efficiency or hepatic vitamin A and carotene retention (Erwin, Elam and Dyer, 1957). Adding 2% of SB resulted in an improved egg production of 11% in laying hens. SB improves feed efficiency, laying frequency, shell quality and egg size and also reduce moisture of the excreta of Leghorn hens (Quisenberry and Bradley, 1964). This experiment was conducted to determine the effect of SB and a synthetic pigment containing lucantin[®] yellow and xanthin on performance and egg quality of laying hens.

Materials and methods Two hundred and fifty six 36-week-old laying hens of the Hy-Line W-36 commercial strain were allocated to eight experimental diets (with four replicates each) in 4×2 factorial arrangement in a completely randomized design. Four levels of SB (0, 1, 2 and 3%) and two levels of pigment were used for 12 weeks of the experiment. Pigment was supplied by 20 gram of lucantin and 20 gram of xanthin. One week before the experiment, the birds were fed dietary treatments for adaptation. Feed and water were provided ad-libitum and a 16:8 light: dark program was followed throughout the 12-week experiment. Egg production and egg weight were determined on 4 eggs randomly collected at the end of each week. Specific gravity (SG) was calculated via following formula: $SG = A/A-B$; A: egg weight in air and B: egg weight in sterile water. Thereafter, these eggs were broken to determine egg weight, shell weight, shell thickness (using micrometer) and yolk colour index (YI, using Roche colour fan). Data were analysed using the GLM procedure of SAS (1999). Duncan's multiple rang test was applied to compare treatments means when $P < 0.05$.

Results Addition of SB decreased ($P < 0.05$) SG and YI when compared to control diet. 40 mg /kg pigment significantly increased SG and, as expected, increased the YI over the control diet ($P < 0.05$).

Table 1 Effect of sodium bentonite and pigment on egg production, egg weight, shell thickness and yolk colour index of laying hens during 36-48 weeks of age

Diet	Egg production%	Egg weight (g)	SG (g/g)	Shell thickness (mm)	Shell %	¹ YI
SB (%)						
0	69.8	66.4	1.07 ^a	0.39	8.4	9.1 ^a
1	65.4	66.3	1.08 ^a	0.40	8.4	8.9 ^{ab}
2	69.2	67.1	1.07 ^a	0.41	8.6	8.8 ^b
3	67.5	67.8	1.05 ^b	0.40	8.2	8.5 ^c
±SEM	1.01	0.74	0.003	0.002	0.12	0.06
Pigment(mg/kg)						
0	66.7	66.9	1.06 ^b	0.40	8.4	6.2 ^b
40	69.2	66.9	1.07 ^a	0.41	8.5	11.4 ^a
±SEM	1.43	0.04	0.005	0.003	0.170	0.08
P values						
SB	0.157	0.713	0.002	0.147	0.560	0.001
Pigment	0.090	0.980	0.021	0.051	0.440	0.001
SB × pigment	0.219	0.926	0.133	0.760	0.918	0.019

^{a, b} Means in each column with different superscript are significantly different ($P < 0.05$)

¹shell weight to egg weight ratio

Conclusions There was significant interaction effect between dietary sodium bentonite supplementation and pigment on yolk colour index. By increasing sodium bentonite into the diet, yolk colour index decreased suggesting when more yolk colour index is required, less sodium bentonite must be used.

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Effects of selenium source on male broiler performance, breast yield and plasma thyroid hormones in different levels of dietary energy

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Introduction Selenium (Se) is a dietary trace mineral for poultry. Organic Se such as selenomethionin (SM) increases plasma Se more affectively than inorganic forms such as sodium selenite (SS) (Payne and Southern 2005). Thyroxine 5'-deiodinase-1 (D1) is a selenoenzyme in liver which converts T₄ to T₃ (main thyroid hormone in poultry) and produces 95% of plasma T₃ (Bianco *et. al.* 2002). This study describes the effects of SM (Biokey[®] Selen) versus SS on growth performance and plasma level of thyroid hormones by using different levels of dietary energy.

Materials and methods A complete random design with a 3×2 factorial arrangement and 3 replicate per treatment was conducted. 450 one-day old male broilers (Ross 308) were placed in 18 floor pens. A 3-phase diet (0-21, 21-42 and 42–slaughter) was used. In each phase, 3 different corn-SBM diets in energy level were used by adding 3 levels of soybean oil (0, 2.5 or 5%) and 0.3 ppm SM or SS was added. All nutrients were adjusted by energy level and met or exceeded the nutrient requirement of broiler.

Body weight (BW) and feed intake recorded weekly and average daily feed intake (ADFI), average daily gain (ADG) and feed conversion ratio (FCR) were calculated. On day 49 two chicks were removed from each pen. Blood samples were collected and pooled by pen. The blood samples were centrifuged for 5 min at 3,000 rpm and plasma were separated. Plasma T₄ and T₃ were determined by using radioimmunoassay (RIA). The data was analyzed by an analysis of variance using the general linear model (GLM) procedure of SAS and means were compared by Duncan's Multiple Rang Test.

Results Se source did not affect BW, ADG and FCR. Soy-Oil increased BW and ADG and decreased FCR. ADFI affected by Se source and energy level interaction (maximum amount was recorded for “+2.5%Soy-Oil & SM”). Breast yield was not affected by Se source or diet energy. Se source increased plasma T₃ and decreased T₄/T₃ ratio only in “+0%Soy-Oil” diets. Diet energy slightly increased T₃ (P=0.089) but did not affect T₄ or T₄/T₃ ratio.

Table 1 Effect of selenium source and diet energy level on growth performance, breast yield (percent of live weight), thyroid hormones and T₄/T₃ ratio¹

Diet level	Energy	Se Source	Final BW(g)	ADFI (g/day)	ADG (g/day)	FCR (Feed/Gain)	Breast Y. (%)	T ₃ (nmol/l)	T ₄ (nmol/l)	T ₄ /T ₃
+0% Soy-Oil		SS	2327.3 ^{b2}	107.4 ^a	50.4 ^b	2.13 ^a	26.3	0.78 ^b	21.25	27.2 ^a
+0% Soy-Oil		SM	2316.3 ^b	102.3 ^{ab}	50.5 ^b	2.04 ^{ab}	26.1	1.55 ^a	20.55	15.0 ^b
+2.5% Soy-Oil		SS	2478.2 ^{ab}	98.5 ^b	53.2 ^{ab}	1.85 ^c	26.0	1.59 ^a	20.52	14.9 ^b
+2.5% Soy-Oil		SM	2629.6 ^a	109.1 ^a	57.4 ^a	1.91 ^{bc}	26.2	1.22 ^{ab}	23.89	19.6 ^{ab}
+5% Soy-Oil		SS	2498.5 ^{ab}	108.8 ^a	54.8 ^{ab}	1.98 ^{abc}	25.0	1.72 ^a	21.90	14.1 ^b
+5% Soy-Oil		SM	2566.0 ^a	107.1 ^a	56.0 ^a	1.92 ^{bc}	25.6	1.77 ^a	24.10	13.9 ^b
<i>P value</i>										
Se Source			0.303	0.560	0.223	0.580	0.579	0.449	0.237	0.349
Energy Level			0.023	0.307	0.022	0.064	0.142	0.089	0.410	0.132
Se × Energy			0.602	0.029	0.508	0.600	0.662	0.092	0.446	0.059
SEM			136.5	4.608	3.02	0.03	0.81	0.41	2.62	5.61

1. BW = 49d live body weight, ADFI = 7 to 49d average daily feed intake, ADG=7 to 49d average daily gain, FCR=7 to 49d feed conversion ratio.

2. Numbers with same letter in each column are not significantly different ($P > 0.05$).

Conclusion According to slight interaction between selenium source and energy level on T₃ and T₄/T₃ ratio, it appears that SM, but not SS, is able to affect plasma T₃ and T₄/T₃ ratio in low dietary energy levels.

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Use of organic acids mixture as an option to replace antibiotic growth promoters in poultry diets

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Introduction Antibiotic growth promotion in agricultural animal production has been practiced for about 50 year in the United States and other countries. Early indications were for a beneficial effect on production efficiency in poultry and swine. There were early concerns about the development of antibiotic resistance in human pathogens resulting in recommendations to ban sub therapeutic use in animal feeds. One of the best alternatives for antibiotic growth promoters are organic acids. Organic acids mixtures have been shown to be an effective alternative to eliminate salmonellas in feeds and chickens. Various organic acid combinations were tested to prevent Salmonella contamination through the ration in day-old chicks, and it was observed that a mixture of formic (70%) and propionic acid (30%), was efficient to eliminate *S. enteritidis* and *S. typhimurium*, both of great importance in human health and increase growth rate in broiler.

Material and methods Five hundred day-old Ross chicks from a commercial incubator were used. Feed and water were supplied ad libitum. The 42-day experimental period was divided into two stages: 1 to 21 and 22 to 42 days of age. Rations were based on yellow corn, soybean meal, and soybean oil. Diets were isoenergetic in both stages and were formulated to meet nutritional requirements established by Rostagno *et al.*(1994). Treatments consisted of the inclusion of a mixture of organic acids (OAs), formic and propionic (at a ratio of 70:30), to replace ground wheat straw as filler at the levels of 0, 0.25, 0.5 and 0.75% and a treatment with 0.15% virginiamycin as growth promoter in the ration. Trial was set up in a completely randomized design, with 5 treatments and four 4 replications of 25 birds. Analyses of variance were run according to the PROC GLM procedure, as well as regression analyses, with the OAs percentages of inclusion as explanatory variables, according to the PROC REG procedure of the SAS program

Results Performance of broiler chicken at 21 days of age was affected by the inclusion of OAs and antibiotic growth promoter in the ration. Live weight increased with increasing incorporation of OA ($P \leq 0.001$), feed conversion ratio, feed intake, and daily gain were affected ($P \leq 0.05$) by the treatments (Table 1), but mortality were not ($P > 0.05$). From 22 to 42 days of age, a difference ($P \leq 0.01$) was observed regarding live weight, feed conversion ratio and mortality. Although Feed intake did not differ ($P > 0.05$) among treatments (Table 1). The mortality observed in the period from 22 to 42 days of age probably resulted from Ascites stress caused by not enough oxygen in the sixth week, and was not related to treatments.

Table 1 - Performance of broiler chickens from one to 21 and 22 to 42 days of age receiving diets with increasing levels of OAs (formic 70%: propionic 30%) and virginiamycin at level 0.15% in Basal Diet.

Treat ¹	0-21					22-42				
	LW ²	FI ³	WG ⁴	FCR ⁵	MR ⁶	LW	FI	WG	FCR	MR
BD	608.0 ^d	950 ^b	572.0 ^d	1.66 ^a	00.00	2077 ^b	33560	1469.5 ^b	2.28 ^a	00.00 ^b
BD+0.25	638 ^c	976 ^b	569.5 ^{dc}	1.63 ^a	00.00	2135.0 ^b	3290.0	1497.5 ^b	2.18 ^{ab}	00.00 ^b
BD+0.50	655 ^{bc}	949 ^b	641.1 ^{cb}	1.55 ^b	2.00	2258.8 ^a	3355.1	1604.6 ^a	2.09 ^b	2.18 ^b
BD+0.75	672 ^{ab}	954 ^b	636.5 ^b	1.50 ^b	00.00	2242.6 ^a	3335	1570.0 ^a	2.13 ^b	1.09 ^b
BD+0.15v	677 ^a	1021 ^a	669.0 ^a	1.53 ^b	1.00	2282.5 ^a	3402.5	1606.3 ^a	2.12 ^b	6.53 ^a
P-value	0.0001	0.0007	0.0001	0.0003	0.1991	0.0001	0.403	0.0006	0.009	0.001
MSE	164	423	370.6	0.002	1.867	1882.92	6215.2	1690	0.01	3.5
SEM	6.4	10.3	9.6	0.02	0.7	21.7	39.4	20.6	0.03	0.9

¹Treatments indicated by basal diet (BD) and its corresponding OAs percentage and virginiamycin (V) at 0.15%. ²Live weight (LW), ³feed intake (FI), ⁴weight gain (WG). ⁵Feed conversion ratio (FCR) and ⁶mortality (MR) means from day one to day 21 and 22 to 42 differ by the duncan test ($P=0.05$)

Conclusion In this study 0.75 % and 0.50 % of OAs significantly improved the performance of broilers over controls and was not significantly different from 0.15 % virginiamycin during the finishing period. These results show that OAs at 0.75 % and 0.50 % could be substituted for the antibiotic virginiamycin as growth promoter.

The effect of betaine supplementing on choline chloride and DL-methionine requirement of male broiler chicks

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Introduction Betain is trimethyl glycerine a methyl donor compound that can reduce other required methyl donors such as methionine and choline. Methionine is an essential amino acid with must not be used for methyl donor in metabolism. Also choline is an important precursor of acetyl choline which is necessary for nervous system and it is better to use choline for other function than methyl donor activity of it. Therefore, the object of this study was evaluation of reducing DL-methionine and choline chloride in broiler diets supplemented with betaine.

Materials and methods In a Completely Randomized Design: 450 one-day chickens were distributed randomly to 6 treatments × 3 replications of 25 male broiler chicks. Strain of the chickens was ROSS308. The experiment carried out for 49 days. Source of betain was betafin which is product of Biochem Company. In first treatment diets were formulated according to NRC (1994) recommendations without betafin (Control group). In second, 4th and 5th treatments; choline chloride were substituted completely by betafin, in 5th and 6th treatments methionine were substituted 7.5% by betafin, in 3th and 4th treatments methionine were substituted 15% by betafin. Metabolizable energy and crude protein were equal in all of experimental diets. Average weight gain, feed consumption, feed conversion ratio and production index were measured and at the end of experiment carcass traits included: dressing percentage, breast efficiency and relative weight of abdominal fat, liver were measured in one bird of each pen. Duncan's analysis was used for comparing between treatments.

Results Mean performance and carcass traits data for the experiment are given in Table 1.

Table 1 Comparison between mean of treatments on performance traits in broilers (0-49 days)

Treatment	weight gain (gr)	feed intake (gr)	FCR (gr/gr)	Production index*	Dressing (gr/100gr)	Breast (gr/100gr)	Abdominal Fat (gr/kg)	Liver (gr/kg)
T1(control)	1935	4378	2.27	153.6	72.6 ^a	28.0	10.21 ^{ab}	1.43 ^{ab}
T2(substituted with choline)	2017	4646	2.3	162.6	73.0 ^a	25.7	10.25 ^a	1.37 ^b
T3(substituted 15%with methionine)	1997	4667	2.34	146.8	68.6 ^b	28.0	9.70 ^b	1.65 ^a
T4(substituted with choline and 15% of methionine)	1961	4656	2.38	162.4	71.0 ^{ab}	28.3	10.00 ^{ab}	1.41 ^b
T5(substituted with choline and 7.5% of methionine)	1923	4473	2.33	143.3	69.3 ^{ab}	26.7	9.80 ^{ab}	1.28 ^b
T6(substituted 7.5% with methionine)	2062	4758	2.31	169.8	73.0 ^a	26.7	10.25 ^a	1.35 ^b
ANOVA								
Mean Square of Error	18027	7799 4	0.005	674	4.33	3.56	536.7	0.0164
Degree of freedom of treatment	5	5	5	5	5	5	5	5
Significant level	0.795	0.589	0.553	0.792	0.084	0.498	0.087	0.057

Mean values with different superscripts on same column are significantly different (P<0.05).

* Production index = (Weight gain(gr)×livability(%))/(Feed conversion ratio × rearing duration(days))/10

Conclusions The results of the present study demonstrates that substitution of total choline chloride and reduction of 7.5% of DL-Methionine had no significant adverse effect on performance, production index and carcass traits.

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Dietary olive oil and soybean oil, when compared with tallow, reduce abdominal fat deposition and plasma cholesterol concentrations in broilers, layers and Thai native chickens

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Introduction Substitution of dietary polyunsaturated fatty acids (PUFAs) for saturated fatty acids (SFAs) reduces the amount of abdominal fat in broilers (Villaverde *et al.*, 2005, Wongsuthavas *et al.*, 2007). The mechanism of the effect of PUFAs is not known (Villaverde *et al.*, 2006), but we have put forward (Wongsuthavas *et al.*, 2007) that PUFAs versus SFAs are preferentially oxidized (Beynen and Katan, 1985) and thereby yield ATP so that carbohydrates are shifted from the oxidative into the lipogenic pathway. The conversion of glucose into body fat is less efficient in terms of energy deposition than is the conversion of fatty acids into fat (Newsholme and Leech, 1984). Monounsaturated fatty acids (MUFAs) are also preferentially oxidized (Beynen and Katan, 1985) and thus should also lower the deposition of abdominal fat. In this study, we tested whether dietary MUFAs indeed reduce abdominal fat mass. Three strains of chickens were fed on diets containing either SFAs in the form of beef tallow, MUFAs in the form of olive oil or PUFAs in the form of soybean oil. De-novo fatty acid synthesis was assessed indirectly by using the concentration of plasma triacylglycerols as indicator.

Materials and methods 7-42 day old male Arbor Acres broiler, layer and Thai native chicks were allocated to one of three dietary treatments and were kept individually in cages. Experimental treatments were subjected to a 3x3 factorial, completely randomized design. Data were subjected to Duncan's multiple range test (Steel and Torries, 1980) using a computer program (SPSS for windows 9.0, SPSS Inc., Chicago, IL, 1998). The level of statistical significance was pre-set at $P < 0.05$. There were 20 birds per strain per treatment. Feed was provided *ad libitum* in the form of meal. Birds had free access to clean water. The experimental diets were formulated to contain 18% crude protein and contained 3% of added fat as beef tallow (BT), soybean oil (SBO) or olive oil (OL). The ingredients were as follows: tapioca starch, 45.52%; soybean meal, 41.05%; rice bran hulls, 4.0%; di-calcium phosphate 3.87%; variable fat, 3.0%; lime stone, 0.50%; DL-methionine, 0.30%; L-lysine hydrochloride, 0.25%; sodium chloride, 0.51% and premix, 1.0%. 42 day old birds were killed at 08.00 am after a three-hour fasting period. Fifteen birds per treatment were randomly chosen for weight measurement of abdominal adipose tissue (from the proventriculus surrounding the gizzard down to the cloaca) and for blood sampling.

Results In the broilers and layers, abdominal fat deposition for the SBO and OL diet was lower than for the BT diet, the difference being statistically significant in the broilers only. In the Thai native chickens the OL diet, but not the SBO diet, reduced abdominal fat. In all three strains of chickens, the feeding of either SBO or OL, when compared with BT, produced a non-significant decrease in plasma triacylglycerols. Plasma cholesterol levels were decreased on the SBO and OL diets when compared with the BT diet, but the lowering did not reach statistical significance in the layers.

Conclusion It is concluded that the feeding of MUFAs in the form of olive oil reduced abdominal fat deposition in broiler and Thai native chicks. The decrease in abdominal fat induced by PUFAs and MUFAs was associated with a systematic, group-mean reduction of plasma triacylglycerols, pointing at a decrease in de-novo fatty acid synthesis.

Table 1 Influence of beef tallow, soybean and olive oil on abdominal fat deposition and plasma lipids concentrations

Items	Broiler			P value	Layer			P value	Thai native chickens			P value
	BT	SBO	OL		BT	SBO	OL		BT	SBO	OL	
Abdominal fat, %	2.90 ^a	2.30 ^b	2.27 ^b	0.05	2.40	2.25	2.28	0.45	1.29 ^a	1.29 ^a	1.16 ^b	0.01
Plasma lipid concentrations (mg/dl)												
TAG	32.5	28.0	29.0	0.18	35.5	27.0	27.5	0.16	35.5 ^a	25.5 ^c	31.5 ^b	0.00
Cholesterol	180 ^a	109 ^b	104 ^b	0.02	127	104	105	0.36	161 ^a	108 ^b	100 ^b	0.01

TAG=triacylglycerol

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Efficacy of clinoptilolite for protecting full-term broilers from aflatoxicosis

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Introduction Aflatoxins (AFs) are toxic metabolites produced by *Aspergillus flavus* and *A. parasiticus*. They are natural contaminants of food and feed stuffs. Their presence in feed depresses body weight, feed intake and feed efficiency (FCR) which has been attributed to reduced protein synthesis, impaired nutrient absorption and reduce pancreatic digestive enzyme production (Allameh *et al.*, 2005). Prolonged exposure to low concentration of the toxin produce severe changes in fat metabolism, bile duct proliferation and an increase in relative weight of liver, which could be attributed to increased lipid deposition due to impaired fat metabolism (Swamy *et al.*, 1998). The purpose of this study was to determine whether a clinoptilolite (CLI, natural clinoptilolite, a proprietary product of Afrand Toska, an Iranian company), would diminish the adverse effects of a feed containing aflatoxin as measured by responses in body weight, feed intake, FCR and liver weight.

Materials and methods In this study day-old male chicks (Ross-308) obtained from a commercial hatchery were placed under continuous lighting with feed and water available for *ad libitum* consumption until they were 6 wks old. There were 4 replicates of 40 chicks for each dietary treatment. The chicks were fed a commercial feed starter (corn and soybean based, 210 g protein, 12.22 MJ kcal ME kg⁻¹) 21 days and thereafter a grower diet (186 g protein, 12.47 MJ ME kg⁻¹) 42 days. Treatments consisted of a group A: basal diet prepared with uncontaminated diet (control); B: control feed plus 20 g/kg CLI, C: feed containing 2 ppm AFs; D: feed containing 2 ppm AFs+20 g/kg CLI. Aflatoxin was produced on rice as a natural substrate toxigenic *A. parasiticus* (NRRL- 2999) was first cultured on rice by inoculating fungal spores (6.5x10⁶-7.0x10⁶) on rice as a natural substrate. Data on weight, food intake and feed conversion ratio were recorded weekly in each replicate group. For total protein and albumin estimation, blood was collected from the wing of the birds (5 birds each replicate) at the end of 3 and 6th week of age, and three chicken from each group were randomly selected and then livers were weighed. Tissue samples were collected and fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 μ and stained by haematoxylin and eosin (H&E). Data were analyzed by the General Linear models procedure of SAS (SAS Institute, 1994).

Results The results showed that CLI improved feed conversion ratio as well as body weight (P<0.05, Table 1). Relative weight (g/100g.b.w.) of the livers in the third group was significantly increased as compared with the control (P<0.05, Table 1). Serum total protein and albumin decreased significantly (p<0.05) in diets contaminated with aflatoxin (Table 1). Histopathologic examination revealed severe fatty change, regeneration foci of liver cells, fibrosis of portal regions and bile ductule hyperplasia. Chickens fed ration containing aflatoxin plus CLI showed no sign of liver injury as judged by light microscopic observations.

Table 1 Mean feed intake, body weight, feed conversion ratio(FCR), total protein,albumin and liver weight.

Treatment	Body w.g (g/d)	Feed intake(g/d)	FCR	total protein (gr/dl)		albumin (gr/dl)		liver wt (g/100g.b.w)	
	d.42	d.42	d.42	d.21	d.42	d.21	d.42	Day 21	Day 42
A(cont.)	38.1 ^{a*}	74.4 ^a	1.95 ^a	2.48 ^a	3.7 ^a	0.72 ^a	1.39 ^a	5.7 ^a	3.4 ^a
B	39.5 ^a	78.8 ^a	1.99 ^a	2.43 ^a	3.7 ^a	0.75 ^a	1.42 ^a	5.9 ^a	3.48 ^a
C	30.2 ^b	69.2 ^b	2.22 ^b	1.75 ^b	2.37 ^b	0.53 ^b	0.75 ^b	7.1 ^b	5.25 ^b
D	35.5 ^a	71.8 ^a	2.05 ^a	2.22 ^a	3.58 ^a	0.67 ^a	1.27 ^a	6.8 ^{ab}	4.1 ^{ab}
s.e.m	0.82	2.5	0.17	0.22	0.3	0.26	0.16	0.35	0.38

*means within columns with no common superscripts significantly (p < 0.05).

Conclusions These results show that the addition of CLI to AFs containing diets effectively diminished the detrimental effects of AF, whilst including it in a diet which did not contain AFs would not have an adverse effect.

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Effect of different energy and protein contents of the diet on performance and growth hormone concentration in the blood of broiler chicken.

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Introduction In all bird species studied, plasma growth hormone (GH) concentrations are high after hatching and during the rapid growth period. Then, plasma GH concentrations decline during the slow growth period to reach low concentrations in adult birds (Harvey *et al.*, 1983). Moravej *et al.* (2006) indicated that low-energy intake could increase FCR and mean plasma concentrations of GH and T3 but could decrease BW and mean plasma concentrations of T4. They also reported that different protein levels during starter, grower and finisher periods in broilers may change the plasma concentrations of GH, T4 and T3 but keeping protein levels nearly constant during these phases of growth did not change ($P<0.05$) these hormones.

Materials and methods 240 day old Ross male broiler chickens were used in completely randomized design with 5 treatment, 4 replicates and 12 chicken in each replicate. Fixed level of dietary energy (12.96 MJ ME/kg) with three different levels of protein offered to the chicken in growth different periods. Protein levels were 22.28, 20.28 and 18.28 %CP in starter period, 19.38, 17.38 and 15.38 %CP in grower period and 17.44, 15.44 and 13.44 %CP in finisher period. Also Fixed level of dietary protein with three different levels of energy offered to the chicken in growth different periods. All chicken fed fixed level of dietary protein (22.28 % CP) in starter period, 19.38% CP in grower period and 17.44 % CP in finisher period with three different levels dietary energy (12.96, 12.12 and 11.29 MJ ME/kg) in growth different periods. The experimental diets base on corn, soybean meal, corn gluten and vegetable oil. Body weight and feed intake were recorded weekly. At 21, 42 and 56 days of age, one male from each replicate that had body weight close to the replication mean was chosen to collect blood sample from the wing veins. Blood samples were assayed for GH concentration by RIA (Radio Immuno Assay). All analyses were conducted using General Linear Model procedures (GLM) of SAS (SAS, 2000), and means were compared by Duncan's test at 5% of probability.

Results Mean feed intake (FI), weight gain (WG), feed conversion ratio (FCR) and growth hormone (GH) concentration data for the experiment are given in Table 1. Results showed that different energy and protein levels affected FI, WG, FCR and GH concentrations. A decrease in dietary protein with fixed level of dietary energy resulted in a decrease in FI ($P<0.01$) and WG ($P<0.01$) and an increase in FCR ($P<0.01$) at 22-42 days of age. No significant differences were observed for FCR between different protein levels at 0-21 days of age and 42-56 days of age. However, lowest protein level had high FCR in these periods. Decreasing the energy content with fixed level of dietary protein resulted in an increase in FI ($P<0.01$), WG ($P<0.01$) but not in FCR at 0-21 days of age. No significant differences were observed for FI and WG between different energy levels at 22-42 days of age. In addition, increasing the energy content improved FCR ($P<0.01$) at 22-42 days of age. No significant differences were observed for FI, WG and FCR at 42-56 days of age. Also, concentrations of plasma GH were affected significantly using different energy and protein levels during different periods. Mean concentrations of plasma GH increased significantly in broilers fed on low energy compared with those fed on high energy during the three periods ($P<0.01$) but differences in GH concentrations were not significant at 42-56 days of age.

Table 1 Mean feed intake(FI), weight gain(WG), feed conversion ratio (FCR) and growth hormone (GH) concentration in different periods.

Treat	0-21 days				22-42 days				42-56 day			
	FI(g)	WG (g)	FCR g/g	GH ng/ml	FI(g)	WG (g)	FCR g/g	GH ng/ml	FI(g)	WG (g)	FCR g/g	GH ng/ml
1	699 ^b	443 ^b	1.58 ^a	8.5 ^b	2228 ^a	1244 ^a	1.79 ^c	6.5 ^b	2133 ^a	771 ^{ab}	2.78 ^a	3.7 ^a
2	709 ^b	398 ^b	1.78 ^a	9.8 ^b	2231 ^a	1150 ^a	1.93 ^b	7.6 ^b	2165 ^a	821 ^{ab}	2.64 ^a	4.7 ^a
3	566 ^c	313 ^c	1.66 ^a	9.9 ^b	1635 ^b	764 ^b	2.14 ^a	7.5 ^b	1658 ^b	575 ^b	2.91 ^a	5.0 ^a
4	824 ^a	525 ^a	1.57 ^a	11.3 ^{ab}	2511 ^a	1194 ^a	2.1 ^a	7.8 ^b	2346 ^a	808 ^{ab}	2.92 ^a	5.2 ^a
5	869 ^a	545 ^a	1.59 ^a	13.2 ^a	2509 ^a	1159 ^a	2.16 ^a	9.4 ^a	2370 ^a	916 ^a	2.72 ^a	6.3 ^a
±SEM	23.7	15.2	0.08	0.86	88.44	44.03	0.03	0.45	100.4	85.5	0.14	0.77

1: 12.96 MJ ME/ kg ,22.28 % CP (0.21 days), 12.96 MJ ME/ kg,19.38 % CP (22-42 days) and 12.96 MJ ME/ kg,17.44 % CP (42-56 days). 2: 12.96 MJ ME/ kg ,20.28 % CP (0.21 days), 12.96 MJ ME/ kg,17.38 % CP (22-42 days) and 12.96 MJ ME/ kg,15.44 % CP (42-56 days). 3: 12.96 MJ ME/ kg,18.28 % CP (0.21 days), 12.96 MJ ME/ kg, 15.38 % CP (22-42 days) and 12.96 MJ ME/ kg,13.44 % CP (42-56 days). 4: 12.12 MJ ME/ kg,22.28 % CP (0.21 days), 12.12 MJ ME/ kg,19.38 % CP (22-42 days) and 12.12 MJ ME/ kg,17.44 % CP (42-56 days). 5: 11.29 MJ ME/ kg, 22.28 % CP (0.21 days), 11.29 MJ ME/ kg,19.38 % CP (22-42 days) and 11.29 MJ ME/ kg,17.44 % CP (42-56 days)

Conclusions Plasma GH concentrations increased in broilers fed on low energy diets compared with those fed on high energy diets Analyses of our data showed that with increased broiler age, mean concentrations of plasma GH decreased.

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Effect of feed restriction and different energy and protein contents of the diet on performance and growth hormone concentration in the blood of broiler chicken

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Introduction Feed restriction during growth increase plasma growth hormone (GH) concentrations observed in domestic birds fed practical diets (Proudman *et al.*, 1981). Nguyen *et al.*, 2005 indicated that lower protein diet (17% CP) reduced live body weight and daily weight gain at early ages. Dietary protein levels higher than 17% CP did not show any significantly effect on growth performance, although increasing dietary protein levels positively improved growth performance and feed utilisation. Dietary energy contents of 12.54 and 13.38 MJ ME/kg did not alter the growth performance of the Betong chicks.

Materials and methods 576 day-old Ross male broiler chickens were used in a 2*2*3 factorials arrangements in a completely randomized design experiment. Each treatment had 4 replicates. Feeding programs (ad libitum and skip-a-day (SAD) feed restriction), energy levels (12.96 or 11.7 MJ ME/kg) and Protein levels (22.28,19.28 and 16.28% CP). SAD treatments were applied during 22-32 days of age. The experimental diets were based on corn, soybean meal and vegetable oil. Body weight and feed intake were recorded weekly. At 21, 32 and 49 days of age, one male from each replicate that had body weight close to the replication mean was chosen to collect blood sample from the wing veins. Blood samples assayed for GH concentration by RIA (Radio Immuno Assay). All analyses were conducted using General Linear Model procedures (GLM) of SAS (SAS, 2000), and means were compared using Duncan's test at 5% of probability.

Results Mean feed intake (FI), weight gain (WG), feed conversion ratio (FCR) and growth hormone (GH) concentration data for the experiment are given in Table 1. In the period of feed restriction (22-32 days of age), the FI and WG of restricted chickens were significantly less than those of ad libitum chickens ($P<0.01$). Also, feed restricted chickens had positively better FCR and higher GH than those of ad libitum chickens ($P<0.01$). FI at 32-49 days of age in restricted chickens was significantly less than those of ad libitum chickens ($P<0.05$), while feed restriction had no effect on WG, FCR and GH at this period. At 0-21 days of age, the chickens fed lower energy diet showed better WG ($P<0.01$), GH ($P<0.01$) and FCR ($P<0.05$) but energy content had no effect on FI. Also, an increase in dietary protein resulted an increase in FI ($P<0.01$), WG ($P<0.01$) and GH ($P<0.01$) and a decrease in FCR ($P<0.01$). At 22-32 days of age, the chickens fed lower energy diet showed better FI ($P<0.01$) and WG ($P<0.01$) but not in FCR and GH. Also, chickens fed high protein diet had higher FI ($P<0.01$), WG ($P<0.01$), lower FCR ($P<0.01$) and GH ($P<0.01$). At 32-49 days of age, the chickens fed lower energy diet showed higher FI ($P<0.01$), WG ($P<0.05$) and FCR ($P<0.01$), while energy content had no effect on GH. Also an increase in dietary protein resulted better FI ($P<0.01$) and a decrease in FCR ($P<0.05$), while protein content had no effect on WG and GH.

Table 1 Mean feed intake, weight gain, feed conversion ratio and growth hormone concentration in different periods.

Treatment	0-21 days				22-32 days				32-49 days			
	FI (g)	WG(g)	FCR g/ g	GH ng/ml	FI(g)	WG (g)	FCR g/ g	GH ng/ml	FI(g)	WG (g)	FCR g/ g	GH ng/ml
Feeding program												
Feed restricted	-	-	-	-	508.8 ^b	279 ^b	1.84 ^b	11.62 ^a	1943 ^b	818.4 ^a	2.40 ^a	5.73 ^a
Ad libitum	-	-	-	-	765.2 ^a	397 ^a	1.98 ^a	8.46 ^b	2064 ^a	854.5 ^a	2.43 ^a	6.22 ^a
± SEM	-	-	-	-	11.12	5.89	0.03	0.44	36.48	21.20	0.04	0.34
Energy (MJ/kg)												
12.96	682.6 ^a	370.94 ^b	1.90 ^a	6.74 ^b	598.4 ^b	319 ^b	1.90 ^a	9.65 ^a	1839 ^b	799.6 ^b	2.32 ^b	6.33 ^a
11.7	712.1 ^a	402.35 ^a	1.82 ^b	9.66 ^a	675.6 ^a	358 ^a	1.91 ^a	10.43 ^a	2168 ^a	873.3 ^a	2.50 ^a	5.62 ^a
± SEM	10.5	5.17	0.02	0.45	11.12	5.89	0.03	0.44	36.48	21.20		0.34
Protein(%)												
22.28	817.8 ^a	511.62 ^a	1.60 ^c	9.74 ^a	790.9 ^a	435 ^a	1.81 ^b	8.81 ^b	2311 ^a	969.8 ^a	2.40 ^{ab}	6.60 ^a
19.28	685.5 ^b	376.15 ^b	1.82 ^b	8.39 ^a	644.3 ^b	353 ^b	1.80 ^b	7.28 ^b	2164 ^b	929.7 ^a	2.33 ^b	5.73 ^a
16.28	588.9 ^c	272.15 ^c	2.16 ^a	6.48 ^b	475.9 ^c	225 ^c	2.10 ^a	14.03 ^a	1535 ^c	609.8 ^a	2.51 ^a	5.59 ^a
± SEM	12.90	6.33	0.03	0.55	13.62	7.21	0.04	0.54	44.69	25.97	0.05	0.42

Conclusions feed restriction increased the GH concentration in broiler chickens, it seems increase plasma GH concentrations birds accompanied by growth delay. The magnitude of the increase in GH concentration reflects the severity of the food restriction. Also, low energy diet and low protein diet increased GH concentration in broiler chickens.

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Effects of substitution of antibiotic growth promoter with organic acids mixture on some serum chemistry in broiler chicken

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Introduction The addition of acids generally lowers the pH and buffering capacity of the diet, reduces pH within the stomach, increases both gastric proteolysis and nutrient digestibility, promotes beneficial bacteria at the expense of pathogenic organisms and decreases intestinal bacterial growth. As a consequence there is an improvement in gastrointestinal health, resulting in enhanced growth performance and improved feed efficiency. Mixture of Organic acids has shown to be an effective alternative to eliminate salmonellas in feeds and chickens. Organic acids reduce the pH in the digestive tract and are able to suppress the growth of undesired micro organisms, mainly in the upper part of the digestive tract depending on the place and rate of absorption. The faster the acids are absorbed the smaller is the pH lowering effect. This effect is directly influenced by the acid binding capacity of the diet. Therefore the aim of this study was to determine that antibiotic growth promoter could be replaced by a mixture of organic acids.

Material and methods Five hundred day-old Ross chicks from a commercial incubator were used. Feed and water were supplied ad libitum. The 42-day experimental period was divided into two stages: one to 21 and 22 to 42 days of age. Rations were based on yellow corn, soybean meal, and soybean oil. Diets were isoenergetic in both stages and were formulated to meet nutritional requirements established by Rostagno *et al.* (1994). Treatments consisted of the inclusion of a mixture of organic acids (OAs), formic and propionic (at a ratio of 70:30), to replace ground wheat straw as filler at the levels of 0, 0.25, 0.5 and 0.75% and a treat with 0.15% virginiamycin as antibiotic growth promoter in the ration. Trial was set up in a completely randomized design, with 5 treatments and four 4 replications of 25 birds. Two birds of each replication selected and killed by decapitation, and a sample of trunk blood was collected quickly (30 s) into vials containing 10 mg sodium heparin and placed immediately into an ice bath. The blood was centrifuged (5000 rpm under refrigeration at 4°C) for 15 min; then, the plasma was decanted and assayed for plasma calcium, phosphorous, glucose, uric acid and cholesterol. Analyses of variance were run according to the PROC GLM procedure, as well as regression analyses, with the OAs percentages of inclusion as explanatory variables, according to the PROC REG procedure of the SAS program.

Results Concentration of serum chemistry of broiler chicken at 21 days of age was not affected ($P>0.05$). From 22 to 42 days of age the only statistically significant change was for calcium concentration to increase in serum above that of the control with all growth promoter additions ($P\leq 0.05$). Phosphorous concentration in serum slightly increase with OAs in the diet, while cholesterol decrease by the inclusion of OAs compared to the control and the antibiotic virginiamycin as growth promoter. In all treatment there was not any significant difference ($P>0.05$) between OAs and antibiotic virginiamycin as growth promoter. (Table 1).

Table 1 Effect of Organic acid mixtures (OAs) on serum chemistry of broiler chickens from one to 21 and 22 to 42 days of age receiving diets with increasing levels of OAs (formic 70%: propionic 30%). And virginiamycin at level 0.15% in Basal Diet

Treat ¹	0-21					22-42				
	Ca ³ Mg/dlit	P ⁴ Mg/dlit	Glu ⁵ Mg/dlit	U.A ⁶ Mg/dlit	Ch ⁷ Mg/dlit	Ca Mg/dlit	P Mg/dlit	Glu Mg/dlit	U.A Mg/dlit	Ch Mg/dlit
BD ²	12.4 ^a	3.8 ^a	231.5 ^a	9.9 ^a	176.1 ^a	8.3 ^b	3.9 ^a	246.3 ^a	10.2 ^a	103.3 ^a
BD+0.25	13.2 ^a	3.9 ^a	223.6 ^a	9.7 ^a	167.9 ^a	9.6 ^a	4.2 ^a	234.8 ^a	10.2 ^a	102.0 ^a
BD+ .50	12.4 ^a	4.3 ^a	221.4 ^a	10.8 ^a	167.5 ^a	9.8 ^a	4.1 ^a	249.8 ^a	11.4 ^a	101.1 ^a
BD+0.75	13.9 ^a	3.1 ^a	221.9 ^a	10.0 ^a	174.5 ^a	10.2 ^a	4.1 ^a	249.9 ^a	11.7 ^a	100.8 ^a
BD+0.15(v)	12.1 ^a	3.9 ^a	225.1 ^a	10.1 ^a	178.6 ^a	9.7 ^a	3.9 ^a	249.6 ^a	11.3 ^a	103.8 ^a
P-value	0.27	0.6	0.574	0.662	0.26	0.0005	0.53	0.5	0.14	0.27
MSE	2.32	0.54	181.90	2.06	385.24	0.42	0.16	361.15	1.95	3.50
SEM	0.54	0.26	4.77	0.51	6.94	0.23	0.14	6.72	0.49	0.66

¹Treatments indicated by: ²basal diet (BD) and its corresponding OAs percentage and virginiamycin (v) at 0.15%. ³Calcium ⁴Phosphorous ⁵Glucose ⁶Uric Acid ⁷Cholesterol. means from day one to day 21 and 22 to 42 differ by the duncan test ($P=0.05$)

Conclusion Most of organic acids improve the condition of the digestive tract and increase the absorption of minerals and organic matter through changes in the micro flora. However in these trials only calcium metabolism seemd to be affected when measured as serum concentrations.

Effect of gamma irradiation on feather meal protein quality for broiler chickens

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Introduction Feathers are a waste product generated in large quantities from commercial poultry processing. Recycling of feathers is an interesting subject among animal nutritionists, because of its potential as a cheap and alternative protein feedstuff. Although feathers are deficient in certain essential amino acids such as methionine, lysine, histidine and tryptophan, they contain other amino acids such as arginine, and threonine (Onifade *et al.*, 1998). Feather waste has been used as feedstuff for poultry and livestock. Without appropriate processing, feather meal has little nutritive value because keratin is not degraded by most proteolytic enzymes. The proteolytic resistance of keratin results from its structural features tight packing of protein chains, hydrogen bonding among polypeptides, and hydrophobic interaction and stabilization of the super-coiled polypeptide chains (Onifade *et al.*, 1998). Many treatments have been developed to increase the digestibility of feather meal and are usually categorized into two groups: hydrothermal treatments and microbial keratinolysis (Onifade *et al.*, 1998). Food irradiation has been recognized as a reliable and safe method for preservation, improve hygienic quality and improve the nutritional value of foods (Diehl, 2002). Lee (1962) observed that sulphhydryl and disulphide groups in proteins are apparently highly susceptible to irradiation and destruction of disulphide bonds improves digestibility of proteins.

This study was conducted to evaluate effect of gamma irradiation on feather meal protein quality for broiler chickens using Protein Efficiency Ratio (PER) and Net Protein Ratio (NPR) bioassays.

Material and methods Samples of feather meal were subjected at ambient temperature to gamma irradiation from a ⁶⁰Co source (NORDION, IR-136, Canada) at Gamma Irradiation Centre, Iranian Nuclear Organization, Tehran, Iran. The applied doses were 10, 20 and 30 kGy as monitored by radio chromic film. In this experiment the protein quality of raw and irradiated feather meals were evaluated using PER and NPR bioassays according to Trevino *et al.* (2000). 80 seven day-old uniform commercial male broiler chicks (Ross 308) were assigned to the dietary treatments in a CRBD experiment with 5 treatments, 4 replicate per treatment and 4 birds in each replicate from 8 to 20 days post-hatching. All diets have formulated according to Trevino *et al.* (2000). PER and NPR were computed by the following formulas:

$$PER = \frac{\text{gain body weight}}{\text{protein consumed}} \quad NPR = \frac{\text{weight gain of test group} - \text{weight loss of protein free group}}{\text{protein consumed}}$$

Results Results presented in Table.1 shows gamma irradiation significantly affected PER values ($p < 0.05$). Feather meal processed at a dose of 10 kGy had the highest PER value (-0.7), but NPR values were not affected by irradiation dose. Although irradiation at 10 kGy was the best dose level for feather meal treatment, this dose level was not significantly different ($p > 0.05$) from control group (0 kGy). Also weight gains of all treatments were not significantly different ($p > 0.05$) from N-free diet and therefore PER values for all treatments were negative.

Table 1 Effect of gamma irradiation on PER and NPR of feather meal for broiler chicks.

treatment	Initial weight (g)	Weight gain (g)	CP consumed (g)	PER	NPR
N-free diet	126.7	-25.08	0.00 ^b	-	-
Feather meal 0 kGy	125.9	-12.19	12.04 ^a	-1.004 ^{ab}	1.117
Feather meal 10 kGy	126.4	-7.87	11.15 ^a	-0.700 ^a	1.579
Feather meal 20 kGy	127.4	-13.45	11.05 ^a	-1.237 ^b	1.086
Feather meal 30 kGy	124.3	-12.39	9.97 ^a	-1.272 ^b	1.277
SEM	0.897	2.438	0.523	0.050	0.054

Means in the same column with different letter are significantly different ($p < 0.05$).

Conclusion Because high levels of energy input in gamma irradiation processing, may destroy some disulphide bonds, a dose of 10 kGy, may be responsible for PER values numerically having increased. At dose levels of 20 and 30 kGy, the high levels of energy input may produce some other tight bonds which are responsible for low PER value at these dose levels. When PER values were adjusted for weight loss of protein free group body weight, no difference were observed between NPR values ($p > 0.05$). It seems that gamma irradiation has little ability to improve the protein quality of feather meal for broiler chicks.

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The effect of reducing fish meal of diets supplemented with DL-methionine and L-lysine hydrochloride on female broiler performance

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Introduction Fish meal is an expensive ingredient in broiler diets and some times it can be harmful if it hasn't produced or stored at suitable condition; On the other hand supplying essential amino acids is more important than using fish meal in diets. Therefore it's possible to reduce the inclusion rate of fish meal by supplementing diets with methionine and lysine as limiting amino acids. The object of this study was evaluation of reducing fish meal in broiler diets supplemented with DL-methionine and L-lysine hydrochloride.

Materials and methods In a Completely Randomized Design: 400 one-day chickens were distributed randomly to 4 treatments \times 4 replications of 25 female broiler chicks. Strain of chicks was ROSS308. The experiment carried out for 49 days. Fish meal level in experimental diets was 0, 1, 3 and 5 percent. Methionine and lysine of diets were equal so that with decreasing in fish meal level, lysine and methionine level of diets were compensated by dl-methionine and l-lysine hydrochloride. Diets were formulated according to NRC (1995) recommendations and Metabolizable energy and crude protein of all of experimental diets were same.

Average weight gain, feed consumption, feed conversion ratio and protein efficiency ratio were measured periodically and at the end of experiment carcass traits included: dressing percentage, breast efficiency and relative weight of abdominal fat, pancreas were measured in one bird of each pen. Feeding cost for one kilogram of live weight in each pen was calculated. Duncan's analysis was used for comparing between treatments.

Results Average weight gain, feed conversion ratio, feed consumption, protein efficiency ratio didn't show any significant difference by reducing fish meal ($P>0.05$). Breast efficiency was significantly more in broilers fed by diets included 3 and 5 percent fish meal than diet without fish meal ($P>0.05$). Therefore it is possible to reduce fish meal in corn based diets without any adverse effect on performance by adding DL-methionine and L-lysine hydrochloride.

Table 1 Comparison between mean of treatments on performance traits in broilers (0-49 days)

Treatment	weight gain (gr)	feed intake (gr)	FCR (gr/gr)	PER (gr/gr)	Dressing (gr/gr)	Breast (gr/gr)	Abdominal Fat (gr/gr)	Pancreas (gr/gr)
Diets without Fish meal	1557	3765	2.41	2.226	0.657	0.279 ^b	0.0191	0.0026
Diets with 1% fish meal	1515	3666	2.42	2.22	0.665	0.294 ^{ab}	0.0197	0.0023
Diets with 3% fish meal	1559	3766	2.41	2.225	0.693	0.312 ^a	0.0148	0.0024
Diets with 5% fish meal	1495	3651	2.44	2.196	0.665	0.304 ^a	0.0131	0.0021
ANOVA								
Mean Square of Error	4268	34261	0.0081	0.007	7.903 ($\times 10^{-4}$)	2.153 ($\times 10^{-4}$)	8.337 ($\times 10^{-8}$)	2.95 ($\times 10^{-9}$)
Significant level	0.451	0.724	0.968	0.952	0.329	0.039	0.62	0.632

Mean values with different superscripts on same column are significantly different ($P<0.05$).

Conclusions The results of the present study demonstrates that it is possible to reduce the fish meal in diets and to attain cheaper feeding cost without any adverse effect by adding essential amino acids.

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Effect of dietary Fermacto® supplementation on performance, transit time of feed and gut microflora of broiler chickens fed diets low in digestible amino acids

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Introduction With increasing concerns about antibiotic resistance, there is a growing interest to find alternatives to antibiotics in poultry production. Diet formulation based on digestible amino acids is an attempt to reduce excreta nitrogen and cost of broiler production. Fermacto®, a microbial feed supplement derived from *Aspergillus mycelium*, is one of the several alternatives that could be used at 1-2 g/kg of diet to improve microbial balance of intestine and performance of young chicks. Fermacto® supplementation is reported to increase uptake of nutrients in marginally deficient amino acids diet. This study was conducted to evaluate the effect of *Aspergillus meal* (AM) inclusion and digestible amino acid levels on performance, transit time of feed (TTF) and ileal lactobacillus population of broiler chicks.

Materials and methods Five hundred forty, day old Ross male broiler chicks were allocated to 9 treatments with 5 replicates of 12 birds each. The chicks were reared to 47 day of age. Three diets were provided to meet recommended (RDAA), 5% and 10% less than recommended digestible amino acids (Creswell and Swick, 2001). The starter (0-21 d) and grower (22-41 d) of each indicated diet was subdivided into three parts and were supplemented with 0.0, 0.0; 0.2, 0.2 and 0.2, 0.1 % of *Aspergillus meal* (AM), respectively. Feed and water were provided ad libitum. A 23:1 h light: dark program was followed. At the end of experiment after 12 h starvation, one chick/replicate close to the average pen weight was decapitated and carcass parts were excised and weighed. Four h after starvation, TTF was determined as the time differences between presenting the marked diet and first appearance of marker in excreta. Lactobacillus population was determined by making a homogenate of one gram ileal content with 9 ml of sterile water and the colony-forming units were determined by plating serial dilutions of the homogenates on lactobacillus MRS agar plates and counting colonies, differentially according to colony morphology, after 48 h of incubation at 37° C under anaerobic conditions (Guban *et al.* 2006). Data were analyzed as a completely randomized design using the GLM of SAS software. The least significant difference test was utilized to compare the treatment means when significant was assessed at $P < 0.05$.

Results *Aspergillus meal* improved ($P < 0.05$) live performance and increased breast and caeca weights as well as ileal population of lactobacillus and TTF. Ten percent reduction in RDAA significantly ($P < 0.05$) reduced final weight and increased FCR of chickens as compared to birds fed RDAA diet. Abdominal fat pad weight increased ($P < 0.05$) as the level of dietary DAA reduced. Chickens fed 10% less RDAA diet had lower ($P < 0.05$) TTF and ileal lactobacillus population as compared to those fed RDAA diet.

Table 1 Performance, carcass parts, transit time of feed (TTF) and ileal lactobacillus counts (ILC) of broiler chicks as affected by *Aspergillus meal* (AM) and dietary level of recommended digestible amino acids (RDAA)

Periods	RDAA			5% less RDAA			10% less RDAA			SEM
	AM (% of diet)			AM (% of diet)			AM (% of diet)			
Starter	0.0	0.2	0.2	0.0	0.2	0.2	0.0	0.2	0.2	
Grower	0.0	0.2	0.1	0.0	0.2	0.1	0.0	0.2	0.1	
Finisher	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
WG (g/b/47d)	2226 ^b	2450 ^a	2447 ^a	2116 ^{bc}	2305 ^{ab}	2290 ^{ab}	2004 ^c	2213 ^b	2205 ^b	74.6
FCR (1- 47d)	2.14 ^b	1.91 ^c	1.92 ^c	2.22 ^{ab}	2.09 ^{bc}	2.11 ^{bc}	2.30 ^a	2.20 ^{ab}	2.21 ^{ab}	0.043
Breast (%) ¹	28.5 ^b	30.1 ^a	30.1 ^a	27.9 ^{bc}	29.2 ^{ab}	29.0 ^{ab}	27.0 ^c	28.5 ^b	28.4 ^b	29.6
Caeca (g)	7.5 ^b	9.4 ^a	9.4 ^a	7.4 ^b	7.9 ^b	7.4 ^b	7.2 ^b	7.7 ^b	7.3 ^b	0.59
Abdominal fat (g)	39.9 ^c	40.4 ^c	41.2 ^c	49.5 ^b	49.3 ^b	50.2 ^b	58.2 ^a	59.5 ^a	57.6 ^a	4.69
TTF(min) on day 16	155 ^b	181 ^a	179 ^a	145 ^{bc}	178 ^a	175 ^a	112 ^d	130 ^c	131 ^c	0.52
TT F(min) on day 32	193 ^b	220 ^a	196 ^b	175 ^{bc}	215 ^a	193 ^b	128 ^d	162 ^c	133 ^d	0.52
ILC, log cfu/g	7.67 ^b	8.38 ^a	8.34 ^a	7.02 ^c	7.69 ^b	7.67 ^b	7.00 ^c	7.66 ^b	7.68 ^b	79.2

^{a-d} Means within each row with different superscript are significantly different ($P < 0.05$)

¹Breast weight: included skinless with bone, given as % of carcass weight.

Conclusions . Feeding 10% less than RDAA diet have reduced performance, TTF, ILC and increase abdominal fat pad of chickens. Fermacto® supplementation at 1 or 2 g/kg of starter and grower diets improved weight gain, FCR, ileal lactobacillus counts of broiler chickens but did not reduce the increase in fat pad weight.

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Efficiency evaluation of alternative antibiotic growth promoters on Iranian Ross broiler performance

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Introduction A number of feed additives including antibiotics have been widely used in the poultry industry for several decades. The manipulation of gut function and microbial habitat of domestic animals with feed additives has been recognized as an important tool for improving growth performance and feed efficiency (Collington *et al*, 1990). Recently, the concerns about possible antibiotic residues and disease resistance have aroused great caution in the usage of antibiotic in the animal industry (Ricke *et al*, 2005). The ban on the use of antibiotic as feed additives has accelerated and led to investigations of alternative feed additives in animal production. The primary alternatives to enhance gut function and growth performance to date include acidification of feed, feeding probiotic organisms, and prebiotic compounds (Patterson and Burkholder, 2003). The main aim of the present research was to evaluate the efficiency of alternative antibiotic growth promoters on growth performance in Iranian Ross broiler performance.

Materials and Methods Chicks fed in a block completely randomized design with 4 replicate pens (15 males per pen). Four dietary treatments used: T₁= Control diet based corn and soybean meal without supplementation, T₂= Control diet+ antibiotic (Virginiamycin) 15 ppm, T₃= Control diet+ probiotic (commercial mixture of lactobacillus, Protexin) 150g/ton diet T₄= Control diet+ prebiotic (commercial mixture, Immnuwall) 450g/ton diet. All meals were made as mash. The male broilers were raised on a battery house under commercial condition and offered ad libitum feed and water throughout the 42 d study. The initial room temperature was set at approximately 32°C and reduced by 2 to 3°C weekly. The performance traits were body weight, average daily gain, average daily feed intake, feed to gain ration (or feed conversion ratio). The traits were measured for individual chicks in the experiment. The data was subsequently analysed by mixed model applying repeated measurements option of SAS programme. Multiple comparisons among treatments were undertaken by Duncan multiple range test.

Results The results of analysis of variance for growth performance traits are shown in Table 1. Supplementation with Virginiamycin, Protexin and Immnuwall significantly (p<0.05) improved Body weight, average daily gain and Feed: gain broilers during the first 42 days when compared with treatment 1.

Table 1: Pair-wise comparisons* of different treatments for growth performance traits

	T ₁	T ₂	T ₃	T ₄	± SEM
Body weight, (g)					
21 d	593.99 ^c	696.27 ^a	680.83 ^a	649.84 ^b	10.21
42 d	1816.03 ^c	2026.95 ^a	1992.50 ^{ab}	1947.61 ^b	24.31
Average daily gain, (g)					
1 to 21 d	26.44 ^c	31.04 ^a	30.53 ^a	28.85 ^b	0.46
21 to 42 d	57.51 ^b	63.80 ^a	61.96 ^a	62.15 ^a	0.95
1 to 42 d	41.98 ^c	47.42 ^a	46.24 ^{ab}	45.50 ^b	0.55
Average daily feed intake, (g)					
1 to 21 d	46.62 ^b	46.84 ^a	46.88 ^a	46.47 ^b	0.10
21 to 42 d	121.78 ^a	122.02 ^a	122.51 ^a	121.69 ^a	0.44
1 to 42 d	84.20 ^{ab}	84.33 ^{ab}	84.69 ^a	84.09 ^b	0.20
Feed: gain					
1 to 21 d	1.98 ^a	1.59 ^b	1.61 ^b	1.74 ^b	0.08
21 to 42 d	2.27 ^a	2.02 ^b	2.14 ^{ab}	2.04 ^b	0.06
1 to 42 d	2.02 ^a	1.79 ^c	1.85 ^b	1.85 ^b	0.02

*Treatments with similar letters were not statistically (p>0.05) different from each others

Conclusions For the traits under consideration, non-significant difference (p>0.05) was found between treatments 2 and 3, suggesting that diet supplementation with Protexin could be used instead of Virginiamycin. The results obtained in the present research also revealed that, for the traits of body weight as well as average daily gain, the treatment 4 had lower values than those of the treatment 2 suggesting that the latter one could be used in replace of the former one if better performance in Ross broilers is expected.

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Effect of sodium bentonite and comparison of pellet versus mash on performance of broiler chickens

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Introduction Pelleting is a processing method that is employed by the feed manufacturing industry to improve farm animal performance. The improvements of pellet on performance have been attributed to 1) decreased feed wastage 2) reduced selective feeding 3) destruction of pathogenic organisms 4) improved palatability. Manufacturers have used various types of pellet binders to increase the durability of pellets. The bentonite type of binders would supply little nutritive value. Bentonite is tri-layered aluminium silicate having sodium or calcium as its exchangeable cation. Feed inclusion is about 1-2%, and this mineral must be hydrated to be functional. The sodium form is the best and hydration of the mineral results in a five-fold increase in weight. During this change, the aluminium silicate layers become separated, and water is attracted to their ionic surfaces creating a 12 to 15 fold increase in volume. The ingredients of bentonite are SiO₂, 66%; Al₂O₃, 16.3%; H₂O (Crystal), 60%; Fe₂O₃, 3.3%; Na₂O, 2.6%; Cao, 1.8%; Mgo, 1.5%; K₂O, 0.48%; Tio₂, 0.12%. This experiment was conducted to study the effect of a local Sodium Bentonite (SB) source and comparison of pelleted and mash diets upon the performance of broiler chickens under the conditions of feed manufacturing plant.

Materials and Methods In this study, 320 day-old male broiler chickens (Cobb strain) were allocated to eight experimental diets in a 4 * 2 factorial arrangements in a randomized block design to evaluate the effect of level of SB inclusion and presentation as pellet or mash on the performance of chickens fed a commercial corn-soybean meal based diet for 7 weeks. Treatments were 0, 1, 2 and 3 percent of SB and mash and pellet forms of the feeds for starter (1-21 days) and grower phases (22-49 days). The starter phase diets contained 20.5% CP and 2850 kcal of ME per kg, whilst the grower phase contained 18.12% CP and 2970 kcal of ME per kg of diet. Body weight gain and feed consumption were recorded at the end of the experiment. Data for all parameters were subjected to an analysis of variance, using the general linear model procedure of SAS (SAS Institute, 2004).

Results Results of feed intake, weight gain and feed conversion ratio (FCR) are shown in Table 1. Pellet – fed birds consumed significantly more feed during the experimental period (P<0.05) and had better weight gain and feed to gain ratio (P<0.05) when compared with mash diets. Feed consumption and weight gain increased when SB added to the diets, particularly at the 3% level of inclusion, but these effects were not significant (P>0.05). Under the conditions of our local feed manufacturing plant, it seems that pelleting the diet compare to the mash and adding SB into the diet at the levels of 1-2% as a pellet binder showed positive effects on performance of broiler chickens

Table 1 Effect of feed form and various levels of Sodium Bentonite (SB) in performance of broiler chickens

Feed form	Feed consumption (g)	Weight gain (g)	Feed to gain ratio
	1-7wk	1-7wk	1-7wk
Pellet	5566 ^a	2764 ^a	2.02 ^b
Mash	5252 ^b	2355 ^b	2.23 ^a
SEM	85.58	33.28	0.024
SB levels (%)			
0	5370	2547	2.12
1	5260	2573	2.06
2	5393	2511	2.15
3	5615	2610	2.16
SEM	121.03	47.07	0.034

^{ab} values with different superscripts in each column are significantly different (P<0.05).

Conclusions As compared to mash the feeding of pellets was associated with a significantly higher body weight due to increased feed intake and improved feed utilisation, but using SB had no statistically significant effect. We found that SB has not negative effect on the performance of chicks and sometimes this material has positive effect.

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Does ascites affect the weight of internal organs in broilers?

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Introduction Ascites is a disease in poultry characterised by increased cardiac output and pulmonary blood pressure, enlargement of the right ventricle and cardiopulmonary dysfunction often leading to death (Julian, 1993). Although the heart, lungs and liver are the main internal organs affected, and spleen weight is a general indicator of stress (Silversides *et al.*, 1997), the gut may also be influenced by this syndrome since it is a metabolically active system that has considerable nutrient and oxygen requirements (Yen *et al.*, 1998). Ascites is a severe cause of loss to the broiler industry in many countries, not only due to high rate of mortality, but also due to reduced weight gain and increased condemnations at slaughter. This study was conducted to investigate weight alterations of heart, lungs, liver, gut and spleen by ascites.

Materials and methods Two hundred fifty day-old Ross male broiler chickens were used in this experiment. Half of the birds were housed in 5 pens (each 1m x.2m per 25 birds) and were reared under normal temperature (NT group) up to 4 weeks of age, then were exposed to constant temperature of 22±1°C until 6 weeks of age. The other half were housed in a room with 5 pens (each 1m x.2m per 25 birds) in a cold temperature condition (CT group). For inducing ascites, the CT group were house at 32°C and 30°C during weeks 1 and 2 respectively. The temperature was lowered to 15°C during week 3 and maintained between 10°C and 15°C for the rest of study (Igbal *et al.*, 2001). Feed and water were provided *ad libitum*. All chickens were fed a mash broiler starter diet (7.65MJ/kg ME and 230g/kg protein) until 21d of age and thereafter a mash broiler grower diet (7.65MJ /kg ME and 210g/kg protein) were formulated to meet or exceed the National Research Council (1994) requirements for broilers. At the end of the experiment (week 6), 5 chickens from each replicate were randomly selected and slaughtered. The heart was removed; the right ventricle (RV) was dissected away from the left ventricle and septum. The weights of right and left ventricles were determined separately. The weights of liver, lung, gut (empty weight) and spleen were also determined. The data were analysed based on a completely randomised design with two treatments with 5 replicates each using the general linear model procedure of SAS. Significant ($P \leq 0.05$) means of treatments were compared using the Duncan test.

Results Table 1 shows the differences of relative organ weights, RV/TV ratio and the percentage mortality between treatments at week 6. As shown in this Table, higher RV/TV and mortality of CT treatment suggested ascites development. There were no significant differences between two treatments for relative lung liver, heart, empty gut and spleen weights.

Table 1 Relative organ weights (to live body weight, %), RV/TV ratio and mortality (%) of treatments.

Treatments	Liver	Lung	Heart	Empty gut	Spleen	RV/TV	Mortality
NT	2.24	0.67	0.67	9.10	1.59	0.20 ^b	2.4 ^b
CT	2.53	0.66	0.73	9.53	1.85	0.29 ^a	8.8 ^a
±SEM	0.980	0.330	0.041	0.388	0.010	0.005	1.790
P value	0.070	0.880	0.368	0.453	0.119	0.0001	0.035

Conclusions The higher RV/TV ratio and mortality rate ($P < 0.05$) of broiler chickens reared at low temperatures (CT treatment) compared with birds reared at normal temperatures (NT) indicates that, in our study, ascites had developed in the low-temperature birds. Despite suggestions that broiler chickens affected by ascites syndrome would have different relative heart, lung, liver and spleen weights than healthy birds the results of our study showed no significant differences between CT (ascites affected) and NT (healthy) birds. Furthermore, although Los Santos *et al.* (2005) observed that under hypoxic conditions, duodenum villus surface area was higher in an ascites-resistant breeding line compared with a susceptible line, no significant differences in empty gut weights were observed in our study..

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The effect of long term treatment of Carbendazim on pre-cocoon parameters of mulberry silkworms (*Bombyx mori*)

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Introduction In sericulture fungicides are applied for two purposes; (1) to heal the fungal diseases of mulberry trees and (2) to prevent and control fungal diseases, such as muscardine, of silkworms (Kuberappa and Jayaramaiah, 1988). Therefore identifying appropriate fungicides with a high efficiency of disease control with low side effects on silkworm are very important. Lin and Tzeng (1994) reported that alpha-DL-difluoromethylornithine (DFMO) treatment showed no deleterious effect on mulberry plants and treated plant parts were safe to use as feed for the silkworms, even immediately after application. The fungicide carbendazim belongs to the benzimidazole class and is recommended to control muscardine and mulberry powdery mildew. Azole fungicides, such as triazoles and imidazoles, are molecules that inhibit ergosterol biosynthesis by preventing the cytochrome P-450 dependent 14 alpha-demethylation of lanosterol. These can also inhibit the cytochrome P-450-dependent metabolism of both endogenous and exogenous compounds (Kuribayashi, 1988). Because carbendazim has recently been introduced to sericulture the present study aimed to investigate the effects of long term application of carbendazim on the biological performance of silkworms.

Materials and methods The eggs of bivoltine hybrid silkworm (103×104), obtained from Iran Sericultural Research Centre (Rasht), were reared under laboratory conditions at 25°C and relative humidity of 75 ± 5%, and photoperiod of 16 h light and 8 h dark. The larvae were fed mulberry leaves (variety Ichenoise) up to the last instar. The study comprised of three treatments, 0 (control), 1 and 2 g/l of carbendazim. The fungicide solutions were prepared from its commercial form, Bavistin®. Fourth instar larvae were divided into three experimental treatment groups with each group consisting of 300 larvae and each replicated three times. Fresh mulberry leaves were soaked in each treatment solution for 10 minutes and then were air-dried for 20 minutes. The treated leaves were then fed to silkworm from the 4th to the 5th instar, once a day. Pre-cocoon parameters such as larval mortality, pupae mortality and larvae weight, determined by weighting five larvae on different days of the 4th and 5th instars, were recorded for each replicate. Collected data were subjected to statistical analysis of variance test using $Y_{ij} = \mu + L_i + e_{ij}$ model (Y_{ij} : record; μ : trait average; L_i : carbendazim level effect e_{ij} : residual effects) to find out the low significant difference between the parameters of the normal control and treated groups.

Results The effect of treatment on the pre-cocoon parameters studied is given in Table 1. There are no significant differences in larval mortality between treatment groups although mortality (%) in the 2 g/l carbendazim treatment was substantially higher than the control (12% and 5.3% respectively). Larval weight was significantly affected by fungicide treatment but there was no difference between the two levels of carbendazim (1 and 2 g/l). The largest difference in larval weight (n=5) was reported for day 7 of the 5th instar (26.34 g and 16.37 g for control and mean of carbendazim treatment respectively).

Table 1 The effect of carbendazim on pre-cocoon parameters of silkworm[†]

Carbendazim Level (g/l)	Larval Mortality (%)	5 Larval weight (g)			Pupa Mortality (%)
		Day4 Instar4	Day5 Instar5	Day7 Instar5	
0 (Control)	5.33±0.03	3.900 ^a ±0.07	18.360 ^a ±0.15	26.343 ^a ±0.21	2.33 ±0.02
1	3.33±0.05	3.246 ^b ±0.04	13.163 ^b ±0.16	16.340 ^b ±0.17	7.40 ±0.08
2	12.0±0.11	3.080 ^b ±0.06	12.990 ^b ±0.12	16.400 ^b ±0.15	8.00 ±0.10
F-value	1.13 ns	24.78 **	57.1 **	736.2 **	0.75 ns
C.V.	107.1	4.42	4.69	1.86	105.44

[†]Means (± SEM) within columns with different letters are statistically significant (P<0.05).

Conclusions In the present study the long-term treatment of mulberry leaves with carbendazim resulted in lower pupal weight. The fungicides Dithane M-45 (mancozeb) and carbendazim have previously been tested for the control of *Beauveria bassiana* and their effects on *Bombyx mori* (Kuberappa and Jayaramaiah, 1988). They reported that dusting was more effective than leaf dipping or spraying and that dusting 5th-instar larvae with 2.0% mancozeb or 0.2 carbendazim daily for five days was most effective in controlling the fungus and also increased some traits of silkworm. In the present study, larvae were fed carbendazim-treated leaves for 11 days and had had considerable effects on pre-cocoon parameters which must be taken into account when using fungicide on mulberry trees in the field. However, it has been reported that the toxic residues on carbendazim-treated leaves only remain for two days following treatment and therefore such leaves could be used for silkworm rearing one week after spraying.

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The effect of Admiral[®] insect growth regulator on the biological traits of first instar larvae of silkworm (*Bombyx mori*)

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Introduction In recent years, many problems have appeared in silkworm (*Bombyx mori*), rearing as a result of pesticide applications to cultivations, especially when mulberry trees are grow next to cultivated plants. Study of insecticides on silkworms includes their toxicity and effects on retardation of development and growth, fecundity, mortality, food utilisation and economic parameters (Bizhannia, 2005). Admiral[®], as an insect growth regulator (IGRs), is a juvenile hormone analogue, with relatively low mammalian toxicity that was first registered in Japan for control of public health pest (Miayamoto *et al.*, 1993). Lately, this insecticide has been used for the control of different pests and much research has been completed on the efficacy of this IGR. Silkworm larvae are extremely susceptible to a number of different IGRs, that at very low doses induces in 5th instar larvae a “Non-Spinning Syndrome” and developmental arrest (Kim *et al.*, 2002; Etebari, 2000). The persistence of insecticides used to control insect pests of mulberry and air drift of pesticides from other neighbouring crops are reported and include study of biochemical changes of silkworm due to insecticide residue (Kim *et al.*, 2002). Also it have been studied its effects on biological traits of silkworm in different stages. The aim of the present study was to study the effect of Admiral[®] residue on biological parameters of silkworm larvae.

Materials and methods The eggs of bivoltine hybrid of silkworm (103×104), obtained from Iran Sericulture Research Centre (Rasht), were reared under laboratory conditions at 25°C and relative humidity of 75±5% and photoperiod of 16 h light and 8 h dark. Immediately after egg hatching, the larvae were divided into six treatment groups with 4 replicates including 100 larvae per each replication. Technical grade Admiral[®], 2-[1-methyl-2-(4-phenoxyphenoxy) ethoxy] pyridine, was prepared and diluted to 0, 1, 10, 75, 150 and 500 ppm concentrations and designated treatment T1-T6 respectively. Fresh mulberry leaves were soaked (100g/l) in each concentration for 10-15 seconds and then air-dried. Larvae were fed the treated leaves on day 1 of the 1st instar only and thereafter were fed with un-treated leaves. Biological and economical traits recorded and the obtained data was subjected to one-way ANOVA of a completely randomised design using SAS (1988).

Results The effect of feeding Admiral[®] treated leaves to larvae on day 1 of the 1st instar on the average of total of feeding period (hours), total of feeding period (hours), total of moulting period (hours), larvae weight on day 1 of the 5th instar (gram), larval mortality (%), pupae mortality (%) and weight of pupae (gram) is shown in Table 1. From obtained results, it was showed that Admiral treated leaves can affected on biological and economical traits, especially in high concentrations such as 150 and 500 ppm. For example, the highest total moulting period belonged to 500 ppm and the lowest one belonged to 0-10 ppm groups.

Table 1 Effect of feeding Admiral[®] treated leaves on parameters of silkworms studied[†]

Treatments	Total of feeding period (h)	Total moulting period (h)	Total larval duration	Larva weight on day 1 of 5 th	Pupae weight (g)	Larval mortality (%)	Pupae Mortality
0 ppm (T1)	424.0 ± 4.17	103.0 ^b ±2.	527.0 ±	4.66 ^b ± 0.46	1.70 ±	3.00 ±	2.78 ±
1 ppm (T2)	413.3 ± 3.86	103.0 ^b ±2.	516.3 ±	5.18 ^a ± 0.37	1.55 ±	8.67 ±	3.72 ±
10 ppm (T3)	391.3 ± 3.42	102.0 ^b ±3.	493.3 ±	5.17 ^a ± 0.42	1.79 ±	9.33 ±	5.16 ±
75 ppm (T4)	422.3 ± 4.19	115.0 ^a ±2.	537.3 ±	4.67 ^b ± 0.46	1.76 ±	6.00 ±	2.05 ±
150 ppm (T5)	412.3 ± 4.89	114.7 ^a ±2.	493.7 ±	5.08 ^a ± 0.51	1.83 ±	8.67 ±	3.45 ±
500 ppm (T6)	410.0 ± 3.96	117.0 ^a ±1.	593.0 ±	5.17 ^a ± 0.28	1.81 ±	4.00 ±	4.14 ±

[†]Mean (± SEM) with different letters are significantly different (P<0.05).

Conclusions The results obtained demonstrated that 1st instar silkworm larvae are sensitive to Admiral[®] since some of the biological traits studied were affected in comparison with the untreated control. A negative effect of Admiral[®] was observed for treatments T4-T6 with corresponding concentrations of 75 to 500 ppm. Therefore, there is a potential risk to silkworms of spraying Admiral[®] in neighbouring fields to horticultures during the rearing period specially, since same concentrations applied in horticulture fields.

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Investigation into the effect of equine head and neck conformation on stride length in walk and trot

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Introduction Conformation of horses has been assessed subjectively for centuries and used as an indicator of future performance and soundness. More recently however, objective methods for assessing conformation have been utilised in research, using quantitative measurements taken of the equine skeleton. Many studies have investigated the effect of different types of conformation on movement in the horse for example Holmstrom *et al.* (1990); however there are none available about head and neck conformation specifically. The position of the head and neck is very important for competition horses as it is used for balance and has been shown to have a significant effect on movement (Gomez-Alvarez *et al.*, 2006). The aim of this study was to investigate the relationship between head, neck and shoulder conformation and stride length in walk and trot.

Materials and methods The study used a quantitative method taken from Holmstrom *et al.* (1990) to assess the influence of head, neck and shoulder conformation on stride length in walk and trot of a group of thirty riding school horses. Self-adhesive circular markers (30mm) were placed on specific anatomical points (cranial end of the axis; proximal end of the spine of the scapular; posterior end of the spine of the scapular; transition between the proximal and middle thirds of the lateral collateral ligament of the elbow joint). Using a digital video camera (Sony DCR-TRV60E) static conformation was recorded with the horses in a standard position. Subjects were recorded in walk and trot, three times consecutively. Clips were downloaded and analysed using two-dimensional motion analysis software (Equinanalysis™). Conformational traits measured were length of head; length of neck; angle of neck; inclination of neck; length of scapular; inclination of scapular and angle of shoulder joint. Stride length was measured using the left forelimb of each horse. Time constraints prevented measurement of both forelimbs. Velocity was also recorded. Statistical analysis was conducted using Minitab Version 14. Differences between velocities of individual horses and repetitions were assessed using General Linear Model. Correlations between conformational traits were assessed using Pearson's correlation. Stepwise regression was carried out to determine which conformational traits were most accurate at predicting stride length. A regression analysis was then performed to verify accuracy and determine a regression equation.

Results No significant correlations were found between neck conformation and either inclination of the scapular or shoulder joint angle (the null hypothesis was accepted). Significant positive correlations ($P < 0.001$) were found between angle of shoulder joint, length of neck and inclination of the neck and stride length in walk (see figure 1). Stride length in trot was significantly positively correlated with inclination of the scapular and inclination of the neck (see figure 2). Velocity did not differ significantly between horses or repetitions ($p > 0.05$).

Figure 1 Fitted line plot showing of length of neck (cm) and mean stride length in walk (cm) showing a positive correlation ($P < 0.001$).

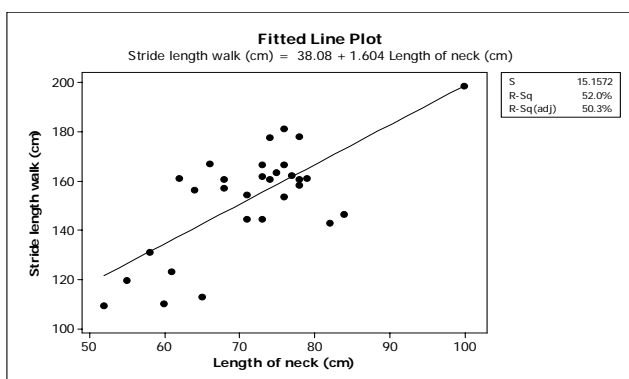
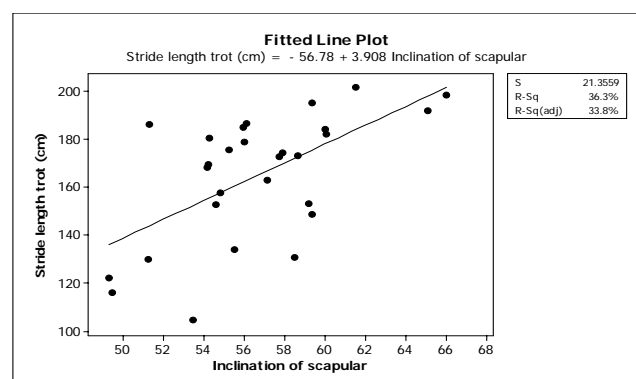


Figure 2 Fitted line plot of inclination of scapular (o) and mean stride length in trot (cm) showing a positive correlation ($P < 0.001$).



Conclusion Head and neck conformation can be used as a predictor of stride length in walk and trot, although it appears that walk can be predicted more accurately. Specifically, a longer neck, greater shoulder angle and more inclined neck were associated with longer stride lengths. This study can be used in conjunction with other conformational studies to provide a useful insight into the effects of different conformational traits on performance and soundness in the equine athlete, ultimately enhancing horse selection and breeding programs.

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Evaluation of a dietary urine acidifier on urinary and reproduction parameters in sows

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Introduction Some of the most common health problems in commercial sow herds are Urinary tract infections (UTI). Prevalence of UTIs ranges from 4 to 40% in sow herds and at least 15% of all sow deaths are generally attributed to cystitis-pyelonephritis (Wegemann, 1993). Examinations of reproductive organs showed a correlation between sow infertility and UTIs. Infections of the urinary tract are known as predisposing factors for inflammations of the uterus. An endogenous mechanism of the sow as protection against bacterial infections in the urogenital tract is the urinary pH, which can be manipulated by dietary means affecting the dietary anion-cation balance (Beker, 1999). The objective of this experiment was to evaluate the effect of a dietary urine acidifier based on a blend of anionic substances, inorganic acids and plant extracts from vaccinium species on urinary and reproductive parameters in sows.

Materials and methods A total of 138 Large White x Landrace breeding sows were split into two groups. During the experimental period (33 days), both control and treated group were fed a conventional lactation diet. The trial group received the dietary urine acidifier Biomin[®] pHD as a top dressing at 20g/sow/day to the conventional diet from day 108 of pregnancy until breeding. Measurements included urinary parameters such as pH (pH), leucocytes (Leuc) and sow reproduction parameters (frequency of repeat breeders, weaning to oestrus interval and incidence of vaginal discharges). Urine samples were taken at day 108 of pregnancy (pH1/Leuc1), one day post farrowing (pH2/Leuc2), 21 days post farrowing (pH3/Leuc3) and one day post breeding (pH4/Leuc4). The samples were collected in the morning between 7 and 9 am. The urinary pH and urine leucocytes were measured with test strips for urinalysis (Combur Test[®] stripes, Roche). Data were analysed as a block distribution model using the ANOVA test analysis procedure of NCSS Statistic Program

Results At the beginning of the trial the overall mean for urinary pH in both groups was 7.2. There was a significant decrease in urinary pH after feeding the dietary urine acidifier compared to the control group (Table 1). There was a significant difference between urine leucocytes concentrations in control and trial group one day post farrowing (Leuc2), with the control group having a higher concentration. Sows of the trial group showed a significant reduced weaning to oestrus interval (4.4 days vs. 6.3 days), a significant decrease in repeat breeding sows (4% vs. 16%) and a significant reduction in purulent vaginal discharges (Figure 1).

Table 1 Effects of a dietary urine acidifier on urinary pH and urine leucocytes concentration

	Control	Biomin [®] pHD	P-value	SED
No. of sows	69	69	-	-
Parity	2.3	2.4	0.705	0.70
pH1	7.2	7.2	0.585	0.145
pH2	7.1 ^a	6.0 ^b	0.031	0.192
pH3	6.9 ^a	5.6 ^b	<0.001	0.173
pH4	7.2 ^a	5.3 ^b	0.723	0.330
pH (2) – pH (1)	0.15	-0.23	0.119	0.230
pH (3) – pH (1)	0.59	-0.46	<0.001	0.230
pH (5) – pH (4)	0.23	-0.63	0.022	0.328

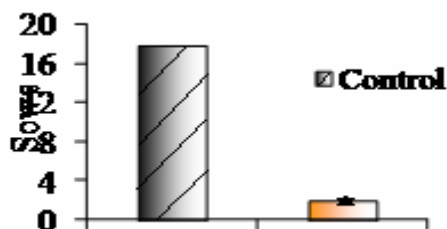


Figure 1 Incidence of purulent vaginal discharges in response to a dietary urine acidifier *P<0.05

Conclusion Biomin[®] pHD reliably reduces the urinary pH at critical times in the reproductive cycle, thus supports the natural defence mechanisms against UTI at critical stages of the reproduction cycle (farrowing and insemination). It offers a possibility of reducing fertility problems associated with uterus infections in sows.

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Qualitative Behaviour Assessment (QBA): a novel method for assessing animal experience

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The assessment of the health and welfare of farm animals is steadily gaining importance, not only from a human health perspective, but also for the sake of the animals themselves. Consumers are increasingly concerned with the effect of industrial farming practices on animal well-being, and so there is a growing need to develop scientifically validated methods which address the welfare experience of animals on farms. Qualitative Behaviour Assessment (QBA) is a novel method developed at Scottish Agricultural College (SAC), designed to make an animal's welfare experience accessible through a 'whole animal' approach. From a 'whole animal' perspective, what animals feel is an integral part of what they do, and so their experience speaks to us through their behaviour, as an expressive 'body language'. By integrating details of an animal's posture, behaviour and of the context in which these occur, we can assess that body language, and judge whether the animal is calm, anxious, enthusiastic or agitated, whether it is generally content or in distress. The question is whether such judgements are reliable, and can be a legitimate part of scientific welfare assessment. Traditionally scientists are taught to be distrustful of applying terms such as 'content' and 'distressed' to animals, regarding use of such terms as 'subjective' or 'anthropomorphic'. However, several years of Defra/SEERAD funded research at SAC have shown qualitative judgements of behaviour to be unfailingly reliable, repeatable, and well-correlated to quantitative behavioural and physiological welfare indicators, both for individual animals and for animals kept in groups (eg Wemelsfelder *et al.*, 2000, 2001; Wemelsfelder & Lawrence, 2001; Rousing & Wemelsfelder, 2006; Wemelsfelder, 2007). Thus, when facilitated by an appropriate methodology, qualitative judgements of behaviour can and do attain scientific validity.

The next step is to develop QBA as a practical on-farm welfare assessment tool. In fact, reading an animal's body language to judge its welfare state is an important aspect of traditional stockmanship – a skill based on practice and experience. Applying QBA on farms could contribute to the formal recognition and extension of this skill, and enhance the communication between farmers and welfare assessors about the well-being of animals on farm. We have recently made a start with developing QBA this way in a commercial context, in a unique collaborative project between SAC, Quality Meat Scotland (QMS), and the Scottish Society for the Prevention of Cruelty to Animals (SSPCA). This presentation will provide an overview of the QBA research described above, and in conjunction with the presentation by Kathy Peebles, will discuss some recent results of our collaborative on-farm work.

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Using Qualitative Behaviourial Assessment (QBA) to measure welfare outcomes for farm assurance

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Since 2002, Quality Meat Scotland (QMS) has been the Meat and Livestock Commission in Scotland. As QMS is both the caretaker for the Scottish red meat assurance schemes which were set up by the red meat industry back in the late 1980's, as well as the owner of the Scotch Beef, Scotch Lamb and Specially Selected Pork brands, it is very well placed within the UK to be able to develop standards that address both consumer and other industry agency concerns.

The QMS assurance schemes follow a whole chain assurance scheme approach. This means that we have six schemes linked as one, from the production of livestock what the animals are fed, how they are transported, where they are sold i.e. through Auction Markets, through to the abattoir. The Scotch Beef and Scotch Lamb brands are PGI (Protected Geographical Indication- a European accreditation) recognised which means that we in Scotland have an assurance chain, which covers the entire life of the animal destined for the food chain, not just the last 90 days of production.

QMS has been aware of the research that Dr Wemelsfelder has been undertaking at SAC, and when the 2005 Farm Animal Welfare Council report called for greater emphasis from assurance schemes to be placed on animal outcomes rather than inputs, it was to Francoise that we turned to see if her Qualitative Behaviourial Assessment (QBA) methodology could be used in the commercial world.

QMS has close links with the Scottish SPCA, through their seat on the QMS Cattle and Sheep Standards Setting Body and the joint on farm inspections that take place with the Society and the QMS farm assessors. Thus it seemed logical to use the Scottish SPCA inspectors to undertake the initial training to be able to use the Qualitative Behaviourial Assessment system on farm during the joint inspection to determine as to whether or not Dr Wemelsfelder's work could be integrated as part of the QMS farm assurance scheme to assess the levels of stockmanship. We are now at the stage where a pilot project is underway in-conjunction with the Scottish SPCA to evaluate the system.

Scotland has been a leader in producing some of the best stock in the world produced by some of the best stockmen in the world. This is an opportunity to be able to quantify those attributes using a unique system through Dr Wemelsfelder's QBA methodology of evaluation.

The value of research at the animal/environment interface

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Introduction Previous generations of animal scientists were extremely successful in increasing livestock production. Surpluses in Europe led us to focus more on other aspects of production systems – such as product quality, environmental effects and animal welfare - in the last 15 years. The recent FAO report ‘Livestock’s Long Shadow’ (FAO, 2006) has reinforced issues about pollution, greenhouse gases and loss of biodiversity associated with the production gains. They estimate that livestock lead to 37% of global methane and 65% of global nitrous oxide, whilst contributing 18% of global climate change. Recent dramatic increases in commodity prices for food, particularly milk protein, remind us of the need to increase productivity to meet the demand from increasing urban populations and compensate for losses to other land uses. The challenge is to develop systems that produce more and better food whilst also reducing pollution potential.

Rapid solutions Reassessment of earlier work on feed evaluation and nutritional requirements, coupled to some rapid experimentation, has provided solutions in some areas. For example, the work of Wu and Satter (2000) led to rapid moves to reduce P levels in dairy diets and thereby reduce P eutrophication. Dilution of maintenance costs means that the excretion of N, P or methane per litre of milk or kg of meat is reduced in high-producing animals – provided that there is no increase in culling or deaths. Thus, the ongoing gradual improvements in areas such as feed quality, animal breeding and animal health have been delivering a corresponding gradual reduction in pollution potential relative to production.

Longer-term solutions There are other situations where production and pollution goals are in direct conflict - and may become even more so as production subsidies are removed. Only new research and sustained implementation can help the industry move to economically viable systems that achieve the high production and low pollution balance. It is interesting to look at how farmers have responded to these questions in systems that are not distorted by production subsidies, such as in New Zealand. The New Zealand dairy industry illustrates the stark conflict between achieving production and environmental objectives. The industry exports around 95% of product and is targeting 3% annual growth. This could be achieved overnight and without supplementary feed if all farmers adopted ‘best’ grazing management practices. However, the practices which lead to elevated yields of high-quality pasture results in pasture with 25 to 30% protein – vastly in excess of requirements. Cows use this feed protein inefficiently and excrete extremely high levels of urinary N, with resultant increased potential for nitrate leaching and nitrous oxide emissions. Further, the use of more expensive low-protein feeds (e.g. cereals) to dilute this protein is not economic when high pasture quality is associated with substitution rates that are close to one.

Solutions to these problems are likely to be medium- to long-term and based on changing forage systems, or plant and animal breeding. High sugar grasses demonstrate the potential to produce high-yielding plants that have more digestible organic matter that is not protein, and thereby reduce urinary N (Moorby *et al.*, 2006). However, progress within immature grass leaves may be limited by the basic relationship between plant growth and the proteins involved in photosynthesis. Solutions may involve plant storage tissues (e.g. maize) or crop fractionation. Animal breeders have recently been selecting for feed conversion efficiency in ruminants (Arthur and Herd, 2005), building on earlier work with monogastrics. Put simply, can we select for cattle that produce more beef for a given amount of feed? The corollary is that the additional beef would be produced with less of the waste products such as urinary N or methane. The fundamental difficulty with this research is lack of rapid phenotypic markers to support molecular or conventional genetics.

Another aspect of solving these production-pollution conflicts is collaboration between animal and soil scientists. Animal and soil scientists working together showed that high N concentrations in urine patches were the main issue for nitrate leaching and nitrous oxide losses. This has led to current promising work using applied or natural nitrification inhibitors to reduce nitrate leaching and nitrous oxide emissions (Di and Cameron, 2002; van Groenigen *et al.* 2006).

Conclusions Animal science has crucial contributions to make across the research spectrum from demonstration to ‘blue sky’. We already has some good solutions for reducing environmental problems linked to increasing production; this needs sustained funding for large-scale demonstration linked to extension. There remain some difficult challenges, particularly where production increases and pollution are diametrically opposed. Progress will come from collaborations with plant and soil scientists, as well as through developing tools for monitoring of systems and breeding for efficiency. There are other areas, such as methane production, where robust solutions remain elusive and new thinking is required.

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The value of research on genetic improvement

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Introduction Modest annual genetic improvements accumulated over a sequence of years can have pronounced impacts on livestock farming industries, and the products generated from them. Genetic evaluation of animals which is a precursor to genetic improvement also provides a platform for tactical use of the genetic variation available at any one time (e.g. identifying easy calving bulls to mate heifers). As a consequence, genetic improvement can have profound implications both within the supply chain and for society in general. Research investment represents one option in the tool kit of government and industry bodies to manipulate livestock product supply chains to benefit their constituents. Industry bodies also have marketing and extension in their toolkit, while governments have the additional option of legislation.

What is special about genetic improvement? Many genetic improvement options are relatively slow to implement, and in extensive livestock farming systems, it can be quite hard to observe noticeable benefits. For extensive livestock industries (sheep, beef cattle and goats), the rate and direction of genetic improvement is controlled and disseminated by a widely dispersed group of farm businesses. For these extensive industries, genetic changes through breed substitution have had at least a comparable impact to that of within breed improvements. In direct contrast, control and dissemination of genetic improvement in intensive livestock industries (poultry, pigs, dairy) is controlled by a relatively small number of large companies operating in a global landscape.

In the developed world, genetic improvement structures and programmes are in place for the majority of farmed livestock systems. Typically, implementation and dissemination of genetic improvement is in the hands of the private sector. However, it is common for databases and genetic evaluation facilities to be supported by, and/or coordinated using funds supplied by industry levies and/or national taxes. Even when these systems have been in place for many years, there can be a wide range in their effectiveness, across breeds, species and countries.

The value of research on genetic improvement Research can impact on genetic improvement in one or more of three main ways; 1.Changes to the rate of genetic progress in economically relevant traits, 2. Changes to the direction of genetic progress such that relative rates of change in contrasting traits are altered and 3. Changes to the rate of adoption of genetically improved animals in commercial farming systems

There is a wide range of potential beneficiaries of research into genetic improvement. These include:

- A. Consumers of the product outside the country of the industry serviced by the genetic improvement programme
- B. Consumers of the product inside the country of the industry serviced by the genetic improvement programme
- C. Members of society with concerns about a livestock industries impact on their own social and physical environment
- D. Product retailers who sell the products of livestock industries to consumers
- E. Product processors who buy animals and animal products from farms and transform them into consumer goods
- F. Farmers who purchase young animals and manage them prior to and during their productive life
- G. Farmers who manage breeding females and their dependant offspring
- H. Suppliers of genetically improved breeding stocks
- I. Suppliers of genetic improvement technologies and services

Historically, both publically and privately funded research on genetic improvement has been targeted at the goals and objectives of beneficiaries B, G and to a lesser extent F. Recent estimates (Amer *et al.*, 2007) of the return on investment into genetic improvement to the extensive farming industries of the UK indicate high returns on investment (average 32% p.a.), but also substantial untapped opportunities due to low adoption rates. Benefits from genetic improvement in the more intensive industries are probably much greater due to higher adoption rates, although because of the globalised nature of the genetic improvement programmes that service these industries, benefits will have largely been to consumers around the world through higher quality and lower prices. For the UK dairy, pig and poultry farming and processing industries, failure to adopt the superior gene stocks would most likely have resulted in a severe reduction in industry competitiveness and would have been catastrophic for them. The impacts on UK consumers would have been much more modest, because of the opportunities to import products from other countries at similar market prices to those paid for domestic products.

Opportunities for government investment into research on genetic improvement Governments contemplating investment in genetic improvement face a complex landscape of investment opportunities. Investments in the pig and poultry genetic improvement components of the supply chain have been private and very effective, initially at lowering the cost of production and improving product quality, and more recently at improving traits linked to animal welfare, so there is limited need for government investment there. Investment in dairy cattle, beef cattle and sheep genetic improvements have focused on simple-to-measure production traits which impact costs of production per unit of output. Because improvements in these same traits also reduce the carbon footprint per unit of product, there could be substantial benefits to society if government investment was to accelerate genetic progress in these traits. A proviso is that sufficient balance must be maintained to ensure that improvements in the primary traits do not lead to deterioration in fitness, health, animal welfare and product quality traits. Investments into new genetic improvement approaches focusing on feed efficiency and more subtle manipulation of ruminant digestion may provide further benefits to society in the future.

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Molecular genetics for livestock improvement – brave new world or false dawn?

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Introduction

Genetic improvement of livestock is largely the result of selection based on estimated breeding values (EBVs) calculated from data on phenotypes and pedigrees. This technology has been very successful without using data on the individual genes responsible for genetic variation. For several years experiments have mapped genes affecting economic traits or quantitative trait loci (QTL) to chromosomal regions using genetic markers. Unfortunately this information has not led to a substantial increase in the rate of genetic improvement.

To overcome the limitations of marker assisted selection, Meuwissen *et al* (2001) proposed “genomic selection” which uses dense markers covering the whole genome so that all QTL are in strong linkage disequilibrium (LD) with at least one marker and consequently nearly all the genetic variance is explained by the markers. Simulation suggested that EBVs with an accuracy of 0.85 could be calculated using only the marker data.

Recently the technology available for mapping QTL and for genomic selection has improved enormously due to sequencing of livestock genomes and commercial platforms that assay 10,000s of single nucleotide polymorphisms (SNPs) at reasonable cost.

In this paper we will consider the effect of this new technology on genetic improvement of livestock.

Genome wide association studies (GWAS)

The high throughput genotyping platforms are being used to conduct GWAS in which 10,000s of SNPs are examined to find those that are associated with a given trait. Commonly >100 SNPs associated with each trait are found and it seems likely that the typical quantitative trait is controlled by 100s of genes. Consequently the variance explained by each gene is small and very large experiments (1000s of animals) are needed to accurately estimate the effect and position of these genes. To date the ability to verify the significant associations in a new sample of animals has been disappointing. This is partly due to lack of power both in the GWAS and in the verification. However, statistically significant associations can be due to relationships among the animals in the experiment and linkage between a SNP and a QTL, but these associations are lost in unrelated animals. A large number of SNPs (>60,000) may also be needed to obtain SNPs in high LD with all QTL. Nevertheless it seems likely that with sufficiently large experiments it will be possible to map and, in the future, to identify the genes responsible for variation in economic traits. However, even without identifying the genes, it should be possible to use the markers to implement genomic selection.

Genomic selection

Using data where many animals have been genotyped for the markers and measured for the trait, a prediction equation is estimated that predicts genetic value from a combination of markers even in animals with no phenotype of progeny. The ideal method of estimating this equation requires a knowledge of the distribution of QTL effects and research is gradually acquiring this knowledge. In the coming years genomic selection will revolutionize livestock improvement. Markers can be assayed at birth and selection decisions made without collecting phenotypes or progeny testing. This will decrease generation intervals and speed up genetic gain. It will become practical to select for many traits which have been difficult to improve in the past such as feed conversion efficiency, disease resistance, longevity and meat quality. However, it is necessary to verify prediction equations on new samples of animals before they are used in practice.

Genomic selection will lead to greater international competition among companies that supply genetic material but will also lower the barriers to entry of new companies. We predict it will dramatically alter the structure of traditional cattle and sheep breeding.

The response to genomic selection will decline more quickly than the response to traditional selection unless new markers are continually being added to the prediction equation.

Other uses of DNA data

SNP assays will also be used in commercial production of livestock to select replacements, allocate them to optimal management and product streams, choose their mates, identify their parentage and trace their products. Consequently DNA profiles on commercial animals will become common and represent a new opportunity to optimize the management of animals based on their genotype.

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Mitigating climate change: The role of livestock in agriculture

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‘There is no bigger problem than climate change. The threat is quite simple, it’s a threat to our civilization’. This statement was made by Sir David King, until December 2007 Chief Scientific Adviser to the UK Government.

The total contribution (both direct and indirect) of agriculture is estimated to be between 17 and 32% of all global, human-induced greenhouse gas emissions (GHG), with direct emissions of methane from livestock estimated to contribute around 7%. Much emphasis has thus been placed on trying to decrease methane emissions, yet the Smith *et al* (2008) identified much greater potential for mitigation of total GHG emissions from livestock production associated with management of grazing land than from decreasing methane production.

Both the UK and the Scottish Governments are currently exploring options for meeting challenging targets for reductions in GHG emissions and a number of reviews have been commissioned to quantify (as far as the data allows) the costs and benefits of a variety of options. Agriculture features prominently within those reviews and livestock farming is one aspect of agriculture.

The objective of this talk builds on, rather than competes with, these studies.

Firstly, it seeks to raise the profile of these studies within the Society, both to avoid duplication through lack of awareness of what has already been done and to open up for constructive debate some of the assumptions on which the estimation of emissions at a global or even a national scale have had to be based.

Secondly, we will explore what the role of BSAS members might be in relation to applying (to the livestock industry) the conceptual framework for considering the costs associated with reducing GHG emissions which was proposed in the Stern review (2006), namely:

1. To reduce demand for emission-intensive goods and services
2. To improve energy efficiency, by getting the same outputs from fewer inputs
3. To switch to technologies which produce fewer emissions and lower the carbon intensity of production
4. To reduce non-fossil fuel emissions, particularly land use, agriculture and fugitive emissions

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The implications of biofuel production on intensive livestock production in the United States

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The United States (US) produces 13.3 billion bushels of corn and 3.2 billion bushels of soybeans annually. Sixty percent of total corn is used in livestock production, with 31% used in beef production, 27% in poultry, 24% in pork, and 15% in dairy. Current US policy dictates that 25% of fossil fuel requirements will be replaced by biofuels by 2025. The entire US corn crop used for ethanol production would replace only 12.3% of the current US fossil fuel demand. Fundamentally, the use of US grain crops will not have a *significant* impact on total fuel use in the near future. Alternative energy sources—cellulosic fermentation products, cropland conversion to sugar cane, increased use of wind, geothermal, or other energy power, or a significant reduction in prices for crude oil will diminish the demand for alternative fuels.

Ethanol and biodiesel production predominate as alternative fuels, with 124 ethanol plants using about 20% of the corn crop in the US and producing 6.5 billion gallons of ethanol. Distillers grains represent a maximum of 40% of the replacement rate of corn in beef cattle, with poultry being only about 5%. Significant costs for shipment of distillers grains from production areas to feeding areas will increase diet costs. Biodiesel production triples annually. Enough virgin soy oil and recycled restaurant grease are available in the United States to provide feedstock for about 1.7 billion gallons of biodiesel per year, or 5% of on-road diesel use. Co-products of this process include glycerol and soybean meal. There will be restrictive future need for soybean meal. Glycerol is currently under study, and studies have shown effective use in dairy cattle, replacing 10% of the corn in beef steers, up to 10% of the diet in pigs, and up to 5% of the diet in chickens. Biodiesel represents the most significant source of alternative fuels in the US. The use of soybeans as the primary feedstock will be under scrutiny because of depleted value of soybean meal for livestock use from overproduction and placing glycerol in a competitive international marketplace.

For the future, use of corn to meet policy requirements for fossil fuel replacement implies 6.6 million additional bushels of corn will no longer be available. Short term implications are higher prices for feed grains as they are diverted to subsidized biofuel production. Higher costs for most livestock and dairy production, because of lower production and increased ownership costs, will make red meats, poultry, and milk less competitive to imports and substitutes. Longer term implications will be from determination of the most economical feedstock for biofuel production. This may include cellulosic fermentation coming on line, and 300 million acres of grasslands could be converted to biomass production. Concentrated dairy and beef feeding operations, including that for the breeding herd, will be similar to current production systems for pork and poultry. The implications to this result on consumer acceptability will be important in the marketplace. The substitution rate of corn with co-products for the dairy and beef industries will be mandate a higher proportion of forage-based diets. Lower milk and meat production could result with reduced energy in the total diet. Complete forage diets may emerge. Supply and demand forces will need to dictate if this result is profitable in the US market since grass-fed meat and milk in the “natural” or organic form resides only in niche markets.

Pigs: Use of biofuel co-products, economics and nutritional limitations

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It is anticipated that there will be a significant increase in the quantity of co-products available from the biofuel industry for use in animal feed. The co-products are the result of either biodiesel or bioethanol production. Biodiesel is produced from oil. One of the main sources of oil is oil seed rape but other oil seeds such as sunflower, Crambe and *Camelina sativa* (Cottrill *et al.*, 2007) may be used. Bioethanol is produced from the fermentation of sugar which is either added directly to the process or obtained from the digestion of starch. The co-products produced include glycerol (glycerine or glycerin) from the production of biodiesel and dried distillers grains with solubles (DDGS) from the production of bioethanol from starch.

The process of producing biodiesel involves the hydrolysis of triglycerides using sodium or potassium hydroxide as catalysts and methanol for methylation. The glycerol produced, which is neutralised using hydrochloric acid, may therefore be limited in its inclusion level due to the high sodium or potassium content. Glycerol may contain levels of methanol but the risk of methanol toxicity is low and limited to meals since methanol will evaporate during the pelleting process. Glycerol is a sweet, high energy liquid that may be utilised by pigs as either a glucogenic or a lipogenic nutrient depending upon the energy status of the animal. During the energy dependant phase of growth glycerol would be metabolised via gluconeogenesis and have a net energy value of 14 MJ/kg. The utilisation of glycerol also requires enzyme activation which is limiting in the pig. High levels of glycerol have a lower energy value because the enzyme system becomes saturated and excess glycerol is excreted via the urine (Doppenberg and van der Aar, 2007). Including glycerol up to a level of 5% in pig diets gives optimum utilisation and increased feed intake and gain have been observed (Kijora *et al.*, 1995). Glycerol has also been demonstrated to reduce carcass drip and cooking losses and increase the levels of C16:0 and C18:1 in the fat via *de novo* synthesis at the expense of polyunsaturated fatty acids.

Where oil seeds are used as the source of oil then oil seed meal is the other co-product produced from the production of biodiesel and rapeseed is often used for this process. The maximum level of inclusion of rapeseed meal in pig diets is primarily dependant upon the level of glucosinolates in the product. The glucosinolate levels may differ between processing conditions used to extract the oil and cold pressed meals have been shown to contain twice the level of glucosinolates as that subjected to heat processing. Trials have shown that '00' rape seed meal, containing 10umol glucosinolate per g, can be included at levels up to 20-25% in finishing pig feeds. It is likely that rapeseed meal will become more cost effective, if the volume of production increases, thus increasing the use of the material in pig feeds.

The main concern with the use of DDGS in pig feeds is the degree of variation in composition and digestibility of nutrients. Variation arises from differences in nutrient analyses of the incoming raw material, amount of condensed distillers solubles added to the distillers dried grains, quantity of starch converted to ethanol by fermentation process and temperature and duration of the drying process (Shurson *et al.*, 2004). Heat processing reduces the availability of amino acids and this will reduce growth unless the inclusion level of DDGS is reduced or the amino acid availability is accurately assessed and accounted for. DDGS also tends to show a high degree of variation in energy content. Phosphorus however has a relatively high digestibility due to the destruction of phytate during processing. Maize DDGS has been used successfully at levels of up to 15% in finishing pig diets but soft carcass fat may be an issue due to the level of poly unsaturated fatty acids in the diet. Wheat DDGS has been used up to a level of 10% in finishing pig diets but a reduction of feed intake has been observed above this level. The use of enzymes may offer a means of increasing the utilisation of DDGS but further investigation appears necessary to determine the type and level of enzyme required. To fully exploit the economic and nutritional value of DDGS in diets for pigs either a rapid method of accurately assessing the nutrient availability is required or processes, which minimise heat damage, should be adopted to ensure the production of a consistent material.

Co-products from the biofuel industry have the potential to be important sources of raw material for the pig industry. In certain areas obtaining further knowledge of how to: 1) improve the production process, 2) rapidly measure the nutrient availability and 3) use new technology to improve the nutrient value, would allow increased utilisation and add value to the co-products produced.

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Ruminants: Use of novel co-products, economics and nutritional limitations, including new technologies for overcoming constraints

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Bio-fuels are probably the most obvious and well known recent development, but whatever the industry the term co-products is now outdated as no factory will ever be constructed without recognition of the value contribution of all products. For example the new Cargill plant in Manchester, that uses wheat as the substrate, produces, starch plus its derivatives, vital wheat gluten, potable alcohol via a joint arrangement with an adjacent company Nedalco, wheatfeed, potentially a liquid feed for pigs and ruminants, plus a ruminant moist feed C☆Traffordgold. They are all essential products to justify capital investment and profitable plant operation.

First generation bio-fuel production is split between bio-ethanol, using grains, sugar cane or beet (or sugar rich derivatives) and bio-diesel, using palm oil, rape seed, soya beans and recovered vegetable oils and tallow. Hence the feed products derived from these processes will vary widely.

In the case of grains for bio-ethanol, these will be distillers grains (with or without the solubles added as these potentially could represent a product in their own right), with a protein content and amino acid composition being a reflection of the grains used plus a contribution from the yeast the extent of which will depend on the actual fermentation process employed, batch or continuous. It is recognised that the particular bio-ethanol production process used can have a marked effect on the nutritional value of these feed products, factors such as, grain quality, grinding conditions, pre-fermentation enzyme and heat treatments to saccharify the starch, the yeast employed, plus the type of evaporation and drying processes.

In the UK wheat will be the grain of choice, and will produce a distillers grain of 32 to 34% protein, on an as received basis, and hence from a protein perspective compete directly with rape seed meal but the higher energy content will command a premium. Rumen protein degradability and digestibility of the by-pass protein, and its' amino acid profile, will impact on its value also and will be affected by the processing conditions. There is very little information on the impact of processing on the nutritional value of wheat distillers for ruminants, the majority of the studies have been undertaken on maize based products and cannot be directly extrapolated to wheat DDGS. Formation of Maillards reactions between the amino acids, especially lysine, and sugars, particularly if they form second stage Maillards will reduce digestibility. It is recognised that the levels of xylose and non starch polysaccharides in wheat DDGS are several times more than those in maize DDGS and hence the potential to form Maillards products is much greater. Current estimates for lysine as a % of metabolisable protein for wheat DDGS are ~ 4.5% compared to soya bean meal at ~ 6.8%. Clearly the feed analysis will be source specific and cannot be treated generically.

The use of sugar cane and beet for bio-ethanol production will produce liquid 'vinasses', a crude EU legal definition, that is rich in fermentation acids, glycerol, soluble protein and NPN, minerals and yeast fragments.

As with the solubles streams from grain based fermentation systems these liquid products appear to have a stimulatory effect on rumen fermentation, probably from the yeast fragments, not live yeast as the distillation process kills the yeast. These effects add to the nutritional value that is not considered in conventional matrix descriptions.

Where oil seeds are used to produce bio-diesel there will be two feed products, the respective protein meals and glycerol the purity of which will depend on the process employed. The impurities will be moisture, organic materials from the oil used that have not been methylated, sodium or potassium as a consequence of hydroxide addition plus a residual amount of methanol. Glycerol yield is approximately 10 to 11% of the oil volume. With the rising cost of cereal grains and other low protein feeds glycerol will provide a valuable means of reducing total dietary protein allow full exploitation of the high protein distillers and oil seed meals from bio-fuel production

Glycerol, a precursor of glucose, has the potential to be a significant part of ruminant rations. The fate of glycerol in the rumen is unclear, early studies indicated it was extensively fermented in the rumen to propionic acid, but more recent from Germany suggest no difference in total tract digestibilities when 15% of the concentrate was replaced with glycerol but only a slight reduction in the acetic acid to propionic acid ratio indicating the rumen may not be as important as first speculated. Our own initial studies suggest that glycerol does not behave in the rumen in a similar manner to cane molasses. Estimates of the energy content undertaken recently in Germany, have suggested an interaction between the concentrate, with high starch diets providing a lower ME than low starch diets, hinting at a rumen involvement.

The volume of rape meal will increase in the EU and with a self sufficiency for soya of only 2.5% there is a growing need to improve the nutritive value of rape meal by removing some of the anti-nutrient factors, e.g. tannins. This may become more economically viable as the differential between rape and soya meal increases.

Clearly, accurate feed material description is fundamental to the full value of the new and novel products being realised.

Nutrient Requirements of Horses: Changes incorporated into the 2007 Horse NRC

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In early 2007, the National Research Council's Nutrient Requirement of Horses – Sixth Revised Edition (commonly known as the 2007 Horse NRC) was published. Given that it had been 18 years since the previous edition had been released, a substantial amount of new information was included which increased the length of the publication over the three-fold from the 1989 version. While a number of requirements or feeding suggestions did not change dramatically, there are some differences worth highlighting. Furthermore, there are some additions to the publication, such as an updated computer program, a section on ration formulation by hand, and a chapter on donkeys and other equids that many individuals will find useful. Sections are also included on feed additives, feeding behaviour, and feeding management of horses.

Those sections will be of interest to many because of the new information provided, but one item likely to have a large impact on the way people think about feeding horses is the change from discussing nutrient requirements on a concentration basis (i.e. a horse needing 10% crude protein) to expressing the requirement on a body weight basis (i.e. 0.266 g crude protein per kg of body weight). This emphasis on the total amount consumed per day versus the concentration in the feed was done for several reasons. First, as many equine diets, especially those formulated for athletic horses, now contain added fat or oil, the total amount of feed consumed by the horse likely will go down as fat and oil provide more calories in a smaller quantity of feed. As a result, a horse on a high fat or oil diet would eat less feed and, if one is feeding nutrients on a concentration basis, the total amount of the nutrient would also decrease. By feeding on a body weight basis, the amount of a nutrient required would remain consistent regardless of the total quantity of feed fed. Additionally, many individuals also only consider the concentrate or grain portion of the diet when evaluating their feeding program. The error in this is that the grain only constitutes part of the ration and the remainder of the diet (usually hay) is often ignored. However, now when one is evaluating the nutrient requirements of a horse, it will be much more logical to consider the contribution of nutrients from all of the feedstuffs and not just the grain.

Another major improvement to the new Horse NRC is a web-based computer program that can be used to determine the requirements of horses of varying sizes and uses. The advantage to having it web-based is that no special software, other than a browser, needs to be installed on one's computer. This greatly increases the accessibility of it. While one limitation of many ration evaluation programs has been that they only work with a PC format, this program has been tested to work with Macintosh computers also. The program will allow the user to input many different variables to calculate the requirements such as weight and age, and other criteria such as whether it is a gestating or lactating mare, or if it is a horse in training. Likewise, though a data base with typical nutrient contents of various feedstuffs is included in the program, users can input their own values if they have the analysis of their feed – thus allowing them to fairly accurately determine if they are meeting the requirements of their animals. One should be able to access this computer program by going to the website of the National Academies Press (www.nap.edu) and typing "horse" into the search engine. On the page that details the publication is a column (currently at the bottom, right-hand side of the page) titled "FREE Companion Computer Program" with a link labelled as "Access the program for free". Clicking on that link should open the program.

The variables of age, weight, use, growth rate, and many others influence the requirements of horses and attempts have been made to quantify the affects these variables have on requirements. A major change in the 6th Edition of the Horse NRC is the recognition that horses vary in their energy requirements based upon temperament and basal activity levels. As a result, the energy requirements for maintenance of a mature horse range from a minimum value that might be appropriate for a docile horse that has little voluntary activity to an elevated value was derived for horses with nervous temperaments that exhibit high degrees of voluntary activity. An average value is suggested for horses that perform moderate amounts of voluntary activity. While it will be up to the horse owner to decide which category their horse might fall into, this range is provided to account for those horses that would be considered an easy- or hard-keeper. While that change accounts for differences in maintenance requirements, another change was to alter the categories used to define the intensity of exercise for working horses. While there previously were three categories of workload (light, moderate, and intense), the new NRC provides four categories (light, moderate, heavy, and very heavy) with each category recognizing an increase in digestible energy requirements (20, 40, 60, and 90% above maintenance, respectively).

Despite the dramatic increase in length of the new NRC, most of the changes to nutrient requirements are not huge. As more research adds to the body of knowledge in horse nutrition, the ability to refine the requirements increases. However, the limitations on conducting horse research make it difficult to be as precise in determining the nutritional requirements of horses compared to other species. Regardless, the emphasis on calculating requirements based on a total amount consumed per day (on a body weight basis) certainly will impact the way many people view feeding horses and will hopefully emphasize the importance of considering the roughage portion of the equine diet and not just the grain portion. In itself, that change should dramatically improve the nutrition of many horses.

Why do we need equine research?

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There are two main reasons why equine research is needed and they are: 1) the horses and 2) the people that work with the horses. A large percentage of equine research is done to improve the health and welfare of the animals. From the determination of nutrient requirements to figuring out ways to prevent disease, a great deal of research focuses on ways to improve the lives of horses. The majority of the rest of equine research deals with ways to aid horse owners. These issues often involve trying to find more cost effective ways of managing animals or examining methods to improve the performance of horses in competition. In a lot of cases, the research serves both purposes. For instance, by finding ways to reduce injuries, suffering by animals decreases and the financial costs or losses associated with injuries are reduced which is advantageous to the horse owner. Furthermore, an injury preventing the horse from being ridden or, even worse, resulting in the destruction of that animal can often be emotionally devastating for those who have great fondness for their animal.

Though most individuals involved in the horse industry can appreciate the importance of equine research, many others find difficulty in placing as great of importance in our work as they might in research being done on animals considered to be food-producing instead of recreational (as is the case with most horses). That has created difficulties over the years in finding ways to fund equine research. As a result, a lot of horse research has required funding from individuals or companies wishing to test the efficacy of a product. While there will always be concerns over potential bias in research being sponsored by someone with a vested interest in the results, such funding is critical to allow us to make advances in equine science. Though research funded in this manner needs to be vetted thoroughly (as should any research), sponsors of this type of research should be lauded. Too often, products are sold to the horse industry without having had any research performed to determine efficacy. As a result, much money is likely wasted. Thus, when a company is willing to proverbially “put their money where their mouth is”, that sponsor deserves to be commended. However, this is true only if the results, regardless of whether they prove positive or negative, are allowed to be made public, especially through publication in a peer-reviewed journal.

Finding funding for equine research becomes more difficult when product testing is not involved. To find funding to conduct research, scientists often depend on one of several options. First, they can seek out philanthropic sources. While not exactly plentiful, there are individuals who wish to donate money to foster research to aid horses or advance knowledge in an area of interest to them – such as in a specific breed or discipline. Another approach is to do research that, while aiding the horse industry, also aids other livestock industries. This type of cross-species approach is more apt to receive funding available to what would be considered the more traditional livestock species. A similar approach is to try and secure funding by using horses as a model for problems with humans. This type of approach probably opens up the most funding opportunities but can be difficult to secure. Horses are an expensive subject to study (compared to animals such as mice and rats that are commonly used), which often limits subject numbers and thus the power of the study. This brings up a final point. Many individuals consider a great deal of equine research to be inferior to research conducted in other species. Typically that is because we are limited in the number of animals we use and also in our ability to do invasive research. While most individuals involved in equine science recognize the importance of horse research, it is critical that we work to establish strong standards so that our research can compare favourably to that done in other species and can be viewed as equally important.

Questioning the purpose of a knowledge transfer strategy

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The discussion will reflect on description, development or implementation of something called a 'knowledge or technology transfer' strategy, backed up by observations and reflections on what leads each of us to change our own behaviours with a view to surfacing perspectives on what the purpose of a knowledge or technology transfer strategy might be.

Recount an inquiry into what a group of people responsible for a knowledge transfer strategy in a part of a UK ministry did, when they did and what they did. This inquiry was conducted by exploring the metaphors used by members of the group and the theoretical entailments of these metaphors.

The question of purpose will be explored, and the case for social learning as a complementary strategy (to the main stream approach) for effecting changes in understandings and practices will be presented.

Who is the competition? Knowledge transfer and the weakness of social networks

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The purpose of this paper is to reflect on the social basis of knowledge transfer, viewing it in a wider context and questioning the usefulness of some of the previous programmes. The empirical basis of this work stems from a series of research projects undertaken in Cornwall over the past 5 years, which considered the impacts of training programmes in the County on land-based businesses. Cornwall has had a very organised agricultural sector that has been determined to use the opportunity of EU Objective one funding to make the sector more competitive, a central part of which has been the provision of training, at a variety of levels and through a number of mechanisms. In the county the English Vocational Training Scheme was altered to allow participants to claim for either childcare or someone to watch over their stock, whilst they took up the training opportunity. It was this scheme that we investigated, initially looking for any new social networks that might have arisen during the training and later how the information from the training was diffused. The resulting social networks and interview data revealed that many of the farm businesses taking part in these projects had a very narrow base of support and advice. Although highly embedded in their communities, with solid local contacts, these were not extensive social networks. The result was that the information about the training was not diffused very widely often going no further than the immediate family. Therefore it was not part of a wider discussion about opportunities or possibilities for the business; rather it was often treated as being of significant competitive advantage and kept close within the key actors in the business. The interview data revealed that the main competitors the business principals believed that they faced were their farming neighbours.

One possibility is that the training being provided through one-day training schemes was of such a low level that it was unlikely to be making a strategic impact on the farm, but was rather of tactical use. For some this was undoubtedly the case, but for others even this small amount of training became the basis of new businesses. Rather I would argue that the research revealed an underlying culture towards knowledge and innovation within sections of the farming community in the County, one that others in the sector had identified and were seeking to change. It also reveals a deficit in the academic understanding of innovation and creativity in rural areas. Work on urban areas, by the Richard Florida and his team, has highlighted the importance of social differences, migration and cultural activity in fostering creative urban areas, with specific technical knowledge or training as of secondary importance. I would argue that often the move to provide knowledge transfer is too narrow an answer to a problem that is broader and more systemic. That rather than looking to provide training or packages of education in order to foster innovation, technical improvement and competitiveness, measures should be addressed to change the cultural milieu in which these businesses land-based or otherwise operate. Broader packages of rural development, fostering diverse and vibrant rural communities may be as important in facilitating the transfer of knowledge and information, as targeted training or education opportunities.

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