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The effect of age and gender on the time taken for horses to learn an operant task

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Introduction The association between the age of a horse and their capacity for learning is understudied despite the large age range of horses used for both competition and leisure purposes in the UK and the considerable number of horses that swap over between different disciplines; for instance 4000 racehorse retire from racing each year and many go on to other careers including 900 each year going on to be polo ponies. A negative correlation between age and learning performance has been reported in test species to-date (Yagi *et al.*, 1988); however relatively few studies have addressed this issue in equines (Madder & Price, 1980; Williams *et al.*, 2004). The aim of the present study therefore was to investigate the association between a horse's age and gender and the time taken to reach a pre-determined criterion during an operant learning task.

Materials and methods All horses were housed at Summerhouse Education and Equitation centre, and received similar management in terms of housing, exercise and nutritional rations daily. Twelve horses; geldings (n=7) and mares (n=5) of mixed breeds were assigned by their ages to one of three age groups (4 to 9 years, 10 to 15 years and 16 to 20 years). Groups were balanced as much as possible for gender and were investigated during 10 days of experimental trials. Experimentation consisted of an initial 10 minute period in which horses were able to habituate to the test situation, equipment and observer. Following this period test subjects received an audible cue of a doorbell and 10seconds within which to respond; with the desired and correct response was to touch a Jolly Ball stable toy suspended in their stable with their nose. A correct response was rewarded using a piece of carrot whilst a negative response was ignored and the test was repeated. Each training session involved 10 repetitions of the task on the same day with horses deemed to have reached criterion level with 8 out of 10 (80%) correct responses gained on two consecutive trials. Subjects who reached this criteria level were said to have learnt the task and their testing ceased. Failure to reach the criteria level after 10 days resulted in being marked not having learnt the task. A one-way ANOVA and Tukey's post-hoc test were used to statistically differentiate between learning capacity of the three age groups. A Spearman Rank Correlation was used to test association between gender and time to learn the task.

Results Nine out of twelve test subjects reached the criterion level within the required 10 day period. A statistically significant positive correlation was seen between age group and the time taken to reach criterion ($R_s=0.805$, $n=12$, $p<0.05$). The time to reach criterion was statistically significantly different between the age groups ($F=12.389$, $df=2$, $p<0.01$). Horses aged between 4 and 9 years were found to be better performers in the operant task in compared to horses aged 10 to 15 and those aged 16 to 20 ($p=0.026$ and $p=0.033$ respectively) (see figure 1). There was however no statistically significant difference found in days taken to reach criterion between horses aged 10 to 15 and 16 to 20. No significant gender differences were reported during this study, but in describing the data it would appear that the percentage success rate of mares was higher in comparison to geldings, with values of 80% and 71.4% respectively.

Figure 1 The correlation between age group and mean ($\pm$ SD) time to reach criterion in days

Conclusion Results suggest that younger horses are apparently more capable of learning an operant task although no significant difference between gender and learning capacity were determined. Further research into gender differences in equine learning incorporating the stallion and taking hormonal influence into account could be very well justified in the equine industry. This research can be considered preliminary research to support existing literature in the field of equine learning with still further investigation to determine whether there is an optimal age for learning and how long the horse has the capacity to continue to learn for.

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Effects of equine back massage pads on stress indicators by the assessment of behaviour, heart rate and salivary cortisol

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Introduction Equine massage therapy is becoming increasingly popular, particularly the use of electronic pads which eliminate the need for a masseur and make the treatment more accessible. Domestic horses live in conditions that are different to which they are naturally adapted. Horses are free roaming, social trickle feeders but modern management houses them in isolation and confinement with little or no social interaction and highly concentrated feeding regimes (Harewood and McGowan, 2005). The inability to carry out these behaviours may lead to stress. Stress may alter the biochemistry of the body and could affect reproduction, growth, metabolism and immune function, which in turn may reduce the performance potential of an individual. Stress can have a negative effect on welfare so it is the responsibility of horse owners to reduce stress. Massage may help in stress reduction due to a similarity to mutual grooming, believed to be a goal directed behaviour and have a reward of stress reduction (McBride *et al.*, 2004). The effect of massage on horses, particularly with an electronic massage pad is not established. The aim of the study was to examine the effect of an electronic pad on the stress perception of domestic horses.

Materials and methods Ten horses were divided into two match paired treatment groups (control and massaged). The massaged group were treated six days per week with the massage pad for six weeks. The massage pad delivers a pre-set timed treatment of 30 minutes. The control group wore a massage pad that was switched off at the same time as the corresponding treated pair. Measurements of frequency of positive and negative behaviour, duration of positive behaviour, average heart rate (HR), standard deviation of HR and salivary cortisol were taken the week before the start of the trial (baseline), weekly during the treatment period and two weeks post treatment at the same time each day. A PolarTM heart rate monitor took continuous readings at 15 second intervals during the treatment. The video camera was set up to film each horse throughout the course of the treatment (30 minutes) and the behaviour was assessed using an ethogram of positive and negative behaviours. Positive and negative behaviours were adapted from McBride *et al.* (2004). An assessment of stress using a saliva sample was collected using a commercially available SalivetteTM oral swab immediately before and after the treatment. Cortisol levels were determined using ELISA technique. General linear models were used to examine the difference between treatment groups for heart rate, behaviour and salivary cortisol.

Results Frequency and duration of positive behaviour was significantly higher ($p < 0.001$) and frequency of negative behaviour was significantly lower ($p < 0.001$) in the massaged group than in the control group during the six weeks treatment. There was a significant difference between mean heart rate ($p < 0.05$) and standard deviation of heart rate ($p < 0.01$) between groups during the six week treatment period (Table 1). Duration of positive behaviour within the massaged group was significantly higher ($p < 0.01$) during treatment weeks than at baseline and post treatment. Frequency of negative behaviour and standard deviation of HR within the treatment group were significantly lower ($p < 0.05$) during treatment than at baseline or post treatment. There were no significant differences between baseline and treatment weeks in the control group. Week number had no significant effect on all measurements ($p > 0.05$). There were no significant differences in the salivary cortisol measurements between groups or during baseline, treatment or post treatment.

Table 1 Mean ($\pm$ se) responses during six week massage treatment (letters denote differences at $p < 0.05$)

	Massage Treatment (6 weeks)	Control (6 weeks)
Frequency of positive behaviour	3.9($\pm$ 0.7) ^a	0.6($\pm$ 0.2) ^b
Frequency of negative behaviour	0.7($\pm$ 0.2) ^a	7.6($\pm$ 1.2) ^b
Mean heart rate (bpm)	33.4($\pm$ 0.7) ^a	35.2($\pm$ 0.7) ^b
Standard deviation heart rate (bpm)	3.4($\pm$ 0.2) ^a	4.4($\pm$ 0.3) ^b

Conclusions Measurements of positive and negative behaviour and heart rate demonstrate the use of an electronic massage pad as a useful tool to significantly reduce stress level indicators in the domestic horse. Week number had no significant effect on all measurements suggesting that results from using a massage pad can be seen as early as the first week. Duration of positive behaviour, frequency of negative behaviour and standard deviation of heart rate returned to baseline levels two weeks post treatment suggesting that the electronic massage pad should be used regularly. This study supports the use of massage therapy to reduce stress but further research is needed to design the optimum massage programme to improve the welfare of the horse.

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Genetic evaluation of UK sport horses for dressage competition

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Introduction Many Northern European countries – including France, Germany, Sweden, Ireland, The Netherlands, Belgium and Denmark – perform genetic evaluations of sport horses (competing in dressage, show jumping or eventing). Publication of estimated breeding values (EBVs) aids selection of animals for breeding to produce progeny with high performance ability. At least 9 of the 10 top ranked horses in the 2008 Olympics individual grand prix freestyle dressage competition were from studbooks that perform genetic evaluations (5 Hanoverian, 2 each of Dutch and Swedish Warmblood). In the UK however, little genetic evaluation of our sports horses has been performed, and EBVs are not currently predicted. The aim of this study was to perform genetic evaluations of horses competing in dressage competitions in the UK.

Materials and methods Results of national and international dressage competitions were obtained from British Dressage. Pedigree data were obtained from the National Equine Database. Data were a selection of all records since 1994, where i) the horse had scored points for performance, ii) percentage (marks awarded from total possible for the test) scoring system was used, iii) the horse had a passport (born/ resident in UK 2004 onward) and iv) it was registered to specific studbooks where pedigree data were available. In the data analysed, 6748 horses competed (376 stallions, 6252 geldings and 120 mares) giving 148997 competitive records. First generation pedigree data were available. Genetic connectedness was reasonable for the sire (mean 1.7 progeny/ sire, 197 sires with >4 progeny and 163 sires also competing). Mixed effects models, using Residual Maximum Likelihood (REML) implemented in the program ASReml (Gilmour et al., 1996) were used to estimate variance components for random effects (additive genetic, horse permanent environment and competition), while simultaneously assessing the effect of fixed variables on the horse's performance. Percentage was used as the performance measure. Horse sex, age (linear, quadratic and cubic covariates), height (linear and quadratic covariates) and competition standard (10 level factor; Advanced, Novice, Freestyle etc...) were included as fixed effects. An animal model was implemented. Heritabilities and repeatabilities were calculated.

Results The variance components for the additive genetic, permanent environment and competition are shown in Table 1. The heritability (s.e.) of percentage awarded was 0.18 (0.018) and repeatability (s.e.) was 0.28 (0.0049). Horse sex, height and age all had significant effects on performance. Stallions performed better than geldings and mares. Performance improved with height from 1.47m (which coincides with the classification of a horse rather than pony) (Figure 1a). Performance improved from the youngest horses to a peak at age 10, and then declined slightly with increasing age (Figure 1b).

Table 1 Variance components for random effects

Variance Source	Component	SE
Horse –genetic	2.120	0.218
Horse – perm environment	1.237	0.194
Competition	2.006	0.036
Residual	6.751	0.036

Figure 1a and b Predicted estimates of percentage at different a) heights and b) ages (+/- 1 standard error bars).

Conclusions The heritability estimate in this population is relatively high compared to other international evaluations, which range from 0.11 (e.g. Hannoverian, Oldenburg and Trakken) to 0.20/ 0.34 (Selle Francais) (Janssens 2008). In contrast to most international evaluations the UK data represents a wide variety of breeds. Breed may account for some genetic variance, producing an upwardly biased estimate of variation within a breed. Stallions are an elite selection of male horses, so better performance is predictable. This sex effect has been demonstrated in other populations. Genetic evaluations of sport horses are required in the UK, to give the breeding market every advantage and to keep up to date with those on the continent. The heritability estimate suggests that selection for performance is feasible in the UK population.

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The relationship between judge assessment of 'collective marks' and overall performance within an elite level dressage competition

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Introduction Dressage competitions consist of the subjective scoring of a number of set movements performed in a specific order within an arena. Hinnemann and van Baalen (2003) suggested that the 'collective' marks awarded are a good judgement of the relative behaviour of a horse performing a dressage test. Those animals that are observed to be more relaxed, calm and appear comfortable in their work are generally awarded higher collective marks. The aim of this study was to examine the relationship between collective marks and overall score (i.e. level of performance) within a dressage competition.

Materials and methods Analysis was conducted on scores obtained from an elite level dressage competition using a set dressage test (FEI Grand Prix - version B 2003). Data collated consisted of all competition score sheets for competitors (n=75). Each competitor was individually scored by five independent, internationally qualified judges situated at specific set locations around the dressage arena. In total, each competitor was scored for 35 separate measures, a sum of 175 actual scores per competitor. These included a total of 31 dressage movements completed in a set order within the dressage test. Additionally judges assessed four further 'collective' scores; paces, impulsion, submission and effectiveness of the rider for the whole test. Each separate movement and the collectives were subjectively scored on a scale between zero (not performed) and ten (excellent).

Comparative analysis using Pearson correlation coefficient between the total collective score awarded (n=4) and grouped movement scores was undertaken. Grouped variables used were: overall score (n=31); total walk score (n=2); total trot score (n=8); total canter score (n=10); total passage score (n=9); and total piaffe score (n=2).

Results Positive correlations were reported for all compared variables. Medium to high correlations were observed between collectives and total movement score (figure one), $r=0.672$, trot score $r=0.742$, canter score $r=0.620$, passage score $r=0.474$ and piaffe $r=0.423$, all at $P<0.05$. A low positive correlation was seen between collective score and walk score $r=0.123$, NS.

Figure 1 Scatter graph collective score versus overall score.

Conclusions Higher collective scores may indicate that the horse is in harmony, calmer and more relaxed. Within the data set studied, higher collective scores were consistently associated with an overall higher test score and higher individual movement scores. The study presented considers a relatively small number of horses and any conclusions drawn need to be mindful of this. However, competitors must consider carefully strategies (within both their warm-up and during competition) that may be employed to attain better collective marks. This will require due thought for the horse's behaviour and welfare.

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Racehorse conformation and its' potential to predict animal performance

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Introduction The conformation of horses can have a large effect on their movement and performance as working animals. This is particularly important for racehorses, as various aspects of body shape can increase or decrease racing performance. For example, steep shoulders are usually a hindrance for jumping, as well as more likely to be connected to lameness problems (Holstrom, 2003). Confirmation will have different effects depending on the type of race the horse is expected to run. A horse which is smaller and more compact will not perform as well in the same races as a larger horse, and the two animals are likely to do well over different styles of jump. For example, a horse with a lower, flatter jump is more likely to do well over hurdles than steeplechase fences, as the latter are larger and more likely to throw the horse off balance (Smith Thomas, 1974). Shoulder angle may prove to be a reliable indicator of success in performance horses, as sloping shoulders enables complete flexibility of the humerus, leading to longer strides and greater speed (Smith Thomas, 1974). Horses with shorter strides have been found to be more prone to lameness in the front legs as the shorter stride length causes the legs to be under more pressure from hitting the ground more frequently (Peham *et al.*, 2001). It has been suggested that the length of the shoulder is also important (Schafer, 1981) and as it is linked to lung capacity - allowing for a deeper ribcage. The aim of this study was to compare conformation and performance in a number of racehorses.

Materials and methods Confirmation data (outside fore shoulder angle and shoulder length, back length, girth circumference (just behind the front legs) and height at the withers) was recorded on a total of 171 animals (flat racers: n=51, National Hunt racers: n=120) across 10 yards. Estimated capacity was calculated using the girth circumference (c) and back length as measurements of a circular cross-section tube (Figure 1) to give a numerical indication of heart and lung capacity (estimated capacity = $(\pi \times (c/2\pi)^2 \times \text{back length})$). Every measurement was taken by the same individual and repeated 3 times to calculate and average. All racing data: rating, race type and age were obtained from the Racing Post website (www.racingpost.co.uk). Regression analysis in Minitab (Version 15) was used to examine the single and multiple relationships between ratings and body confirmation data.

Figure 1 A circular cross-section tube shape to calculate estimated capacity $\pi \times (c/2\pi)^2$ back length

Results Analysis of the data showed that the relationships between rating and confirmation was particularly strong in the flat racers but weak in the National Hunt racers and therefore the flat racers are of greater interest and relevance, hence the results discussed here are for flat racers only. Age was significantly related to rating but had a low accountable variance ($P < 0.001$, $R^2 = 21\%$) so consequent analysis was performed on subgroups selected by age as well as type of competition. Height was correlated to rating in 2 and 4 year-olds ($P < 0.01$, $R^2 = 53\%$; $P < 0.05$, $R^2 = 43\%$ respectively) whereas shoulder angle, shoulder length, back length, girth circumference and estimated capacity were not, however none of the measurements taken were correlated to rating in 3 year-olds. Rating was related to girth circumference and estimated capacity ($P < 0.05$, $R^2 = 38\%$, $P < 0.05$, $R^2 = 39\%$) in the oldest group comprising of 5, 6, 7 and 8 year olds. When including multiple measurements, rating was strongly related to height, shoulder angle and shoulder length ($P < 0.05$, $R^2 = 80\%$) in four year-olds and to height, shoulder length, back length, girth circumference and estimated capacity ($P < 0.01$, $R^2 = 96\%$) in the oldest group.

Conclusions Body shape is important in flat racers only where a higher rating is achieved by the larger horse which is in agreement with Smith Thomas (1974), however the age of the animal was important. This disagrees with Holstrom (2001) who believed that the conformation – performance relationship was set as young as 8 months of age. This work represents a snap shot of data collection at one time point, further research is necessary, however ultimately if this approach worked the results could be used to predict racing success of individual animals.

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Fit and fat; a horse carer's perspective

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Introduction Obesity in horses and ponies, is according to many equine related charities (Blue Cross, BHS) becoming an increasing welfare problem. Recent studies have linked equine obesity and insulin resistance with development of debilitating laminitis (Vick *et al.*, 2007). Poor nutritional management and sedentary lifestyles are to blame for the growing proportion of obese horses (Buff *et al.*, 2005). The horse carer is responsible for the health and welfare of the horse and as such should monitor the weight, condition and exercise regime to ensure appropriate fitness levels to maintain health and performance. Indeed, Johnson (2002) reports that certain management practices tend to promote the development of obesity in mature horses as they enter their teenage years. Horse owners must therefore consider management practices throughout the horse's life, as the provision of starch-rich and fat-supplemented rations to healthy horses that are relatively inactive may promote the development of obesity increasing the risk of laminitis (Johnson, 2002). The aims of this research were to ascertain the condition score and fitness levels of horses and ponies when diagnosed with laminitis, as reported by the horse carer and to gain insight into how these factors may influence the health and welfare of the domestic horse.

Materials and methods An online questionnaire was open for completion for a one month period and received over 600 responses, of which 569 completed the responses concerned. Respondents were asked to complete the survey only if they were familiar with an animal resident in the UK that had been diagnosed (not necessarily by a veterinary surgeon) with laminitis during the previous twelve months. A wide range of questions to gather equine demographic and management related data were included within the online survey. Condition score was assessed (by the respondent) using the Carroll and Huntingdon (1988) 0 to 5 method, with diagrams and written descriptions. Respondents were asked to categorise the animals' fitness levels and work (use) at the time of diagnosis using detailed given descriptions to assist them. The data were categorical and analysed using chi squared tests.

Results In relation to the condition score 60.3% of subjects were categorised as moderate or good, however 34.4% were fat or very fat, a significantly non uniform distribution (X^2 , $df=5$, $p<0.001$). The modal fitness category was 'hacking fit' with 40.5% of subjects and whilst 28.9% of subject were unfit, 13.9% were categorised as very fit, a significantly non uniform distribution (X^2 , $df=3$, $p<0.001$). The association between condition score and fitness level is shown in table 1, and was statistically significant (with very poor and poor combined to satisfy test requirements) (X^2 , $df=12$, $p<0.001$). Fitness levels were compared against the work that the subject was undertaking at the time of diagnosis and 29% of subjects did not appear to have an appropriate level of fitness for the work being undertaken. 10% of subjects were reported as having greater fitness levels than their work would suggest e.g. a retired unriden companion categorised as very fit. 19% of subjects were undertaking work that would require a greater level of fitness than was reported e.g. hunting whilst hacking fit.

Table 1 Frequency of responses

Condition score	Unfit	Hacking fit	Fit for light competition	Very fit	Total
0 = very poor	2	1	0	2	5
1 = poor	5	5	4	3	17
2 = moderate	28	31	13	13	85
3 = good	57	116	54	35	262
4 = fat	62	72	16	14	164
5 = very fat	13	7	2	14	36
Total	167	232	89	81	569

Conclusion It is not surprising that a large proportion of horses and ponies when diagnosed with laminitis are reported to be fat or very fat, in fact this figure may have been expected to have been even higher. What was surprising was that only 28.9% of these horses were unfit and some were very fit. However of most concern was the categorising, of horses as very fat and very fit. A horse with a condition score of 5 is at severe risk of health complications and categorising this animal as very fit shows a complete lack of understanding of 'fitness levels' and/or the condition scoring. Whilst condition scoring has a recognised and repeatable method that a horse carer can apply, an equivalent fitness assessment is not currently available. There are serious health and welfare concerns relating to the owner's expectation to be able to use these animals to complete high intensity activities. The mismatch between use and fitness levels across the sample, combined with the over-representation of fat animals, underline the challenge to the equine industry of increasing the education of horse carers.

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An investigation into the weaning practices used within the UK equine breeding industry sector

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Introduction Weaning occurs during the development of the young horse, involving maternal deprivation, abrupt nutritional challenges and social changes (Nicol, 1999) in both the free-living and domestic horse. Both psychological and physical aspects of well being can be impacted on if weaning is received with a negative response by the individual (Hoffman *et al.*, 1995). Weaning can therefore evoke both short term and long term responses. Short term includes depressed immunity, inappetance and increased cortisol and adrenal responses. Long term changes include locomotory or oral stereotypies development (Waters *et al.*, 2002), reduced ability to interact with other equines (Weeks *et al.*, 2000), and affects on the human-horse bond and trainability (Søndergaard and Ladewig, 2004). There is an abundance of research discussing the advantages and disadvantages of different foal weaning practices and which is best (Waran *et al.*, 2008). Parker *et al.*, (2008) examined horse breeding management in Europe, North America and Australia. The USA sample population were weaned at a much earlier age with a greater percentage displaying abnormal behaviours. However there is little research to suggest which weaning methods, such as gradual or abrupt, are actively used or favoured within the horse breeding industry. The current study therefore sets out to investigate which method of weaning is most common within the UK equine breeding industry.

Materials and methods A pilot study was conducted to develop the research questionnaire, and to determine interpretation of the questions by participants in terms of content and structure. Once final amendments were complete, six hundred and forty eight surveys were sent out via email to a number of UK studs. Respondents were selected from breed society websites and mainstream equestrian websites advertising the stud. One hundred and eight surveys from individual studs were returned successfully, resulting in a 16.6% response rate. The final questionnaire contained twenty multiple choice questions, each with additional comments boxes. All questionnaires were submitted anonymously and respondents were offered the opportunity to receive a summary of the results. All the survey data was analysed using SPSS; Statistical Package for the Social Sciences. Frequencies and percentages for each question answer were calculated and data was split by weaning methods; gradual, abrupt and dependent on circumstances groups. Data was also split into small stud and large stud groups. Comparisons of answers from small and large studs were drawn to highlight trends between groups. Chi squared tests were performed where possible to compare data and assess significance of results. In cases where the variables create a 2x2 contingency table the Yates correction value is used to compensate for chi squared overestimating values. In some cases a small percentage of the cells did not meet the expected count so a Fisher's exact probability test value was used.

Results Eighty three percent of studs reported to breed foals annually, regardless of how many foals were bred or the size of the stud. Abrupt weaning methods were used by eighty one percent of studs. No significant relationship was found between weaning method and stud size ($P>0.05$). Creep feed was utilised for the majority of foals pre-weaning. Age, and mare-foal interactions were the main reason for choosing time of weaning regardless of stud size, however the trend was not strong enough to be significant ($P>0.05$). No significant relationship was found between weaning method, stud size and post-weaning housing ($P>0.05$). Foals were kept mostly indoors post-weaning, being assigned to groups made up of playmates as opposed to same age or sex. A correlation was found between stud size and housing of foals in groups, pairs or individually. The majority of studs reported that the method chosen was, in their opinion, best for the foals. Gradual techniques were highly variable ranging from several separations over one day, to separations every few days for up to a month.

Conclusions No significant relationship was established between method of weaning used and size of stud, justification for time of weaning or method of housing post-weaning, however the study highlighted that the majority of studs utilise the abrupt weaning method chosen as this is thought to be the best option for foal welfare. Larger studs had more group housing allowing social interaction and increased foal welfare. Smaller studs tended to house in pairs or individually, where potential stress issues could arise. The majority of studs used creep feeding prior to weaning which has been suggested to contribute to abnormal behaviour development as the foal matures. Results suggest that studs prefer the abrupt weaning method, although the technique varies between studs and their facilities, time, mare/foals reactions, discipline horses used for and finance.

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Social play and its initiation in an established group of young domestic horses

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Introduction Although ethograms of social play behaviour have been formulated (McDonnell & Poulin, 2002; Zharkikh, 2003) few studies focus on equine social play (Christensen *et al.*, 2002). Social play studies have largely focussed on the time budgets and generalised interactions between herds (for example Boyd, 1988) rather than specific social behaviour sequences between limited numbers of horses. However, by observing pairs or small groups of animals, much may be learned about the dynamics of social communication in a particular species (Kalmus, 1969; Poole, 1972). Although play appears to be considered as either functionless or as serving different roles depending on species, age and even sex of individuals Burghardt (2006) speculated that 5 selected advantages in the performance of play within the behavioural repertoire of an animal existed, control, adaptive function, development, evolution and private experience. Further knowledge of equine social play may be of benefit to equine welfare and performance. The aim of the current study was to describe and quantify the involvement in social play and its initiation within an established group of young horses.

Materials and methods An established grazing group of 8 horses aged between 18 months and 3 years were observed in a single 8 acre pasture enclosure. The group had been managed together at pasture for over 9 months. The enclosure possessed abundant, evenly distributed, grass and water and mineral blocks were available *ad libitum*. Human interference was minimised and horses received no additional food (Christensen *et al.* 2002). Observations were made from outside the enclosure perimeter following a period of acclimatisation. Behavioural data were collected during 100 hours of direct observation using continuous behaviour sampling for 2 hour periods between 06.30 and 08.30 hours. Social play behaviours were recorded using McDonnell and Poulin's ethogram (2002) which includes social object play, social locomotory play, play fighting and play sexual behaviour, and by definition involves more than one participating horse. A social play bout was recorded as commencing when one of these behaviours was exhibited by a horse, the initiator, and ended when a behaviour was observed that was not classified by McDonnell and Poulin (2002) as being a social play behaviour.

Results A total of 134 social play bouts were noted, giving a mean frequency of 1.34 social play bouts per hour. Social play bouts were observed in 71 of the 100 hours of observation. Therefore during 29 hours of the study, no social play was displayed. Of the 134 social play bouts observed 115 were initiated by an individual horse and 19 by groups of horses. The number of play bouts initiated by individual horses ranged between 3.0% and 50.7% and this number significantly varied between individuals (X^2 , $df=7$, $p<0.001$). All horses were solely responsible for the initiation of at least 2 social play bouts (mean $\pm$ SD, 14.38 ± 15.14), however 2 horses were not part of groups initiating play (see table 1). The mean ($\pm$ SD) number of group initiations individuals participated in was 5.75 ± 7.21 and significantly varied between individuals (X^2 , $df=7$, $p<0.001$). Of the 19 social play bouts initiated by groups, 3 were initiated by a pair of individuals, the majority (12) were initiated by 3 individuals, 2 by 4 individuals, 1 by 5 individuals and 1 by 6 individuals. H3 initiated 50.7% of all social play bouts and initiated the most social play bouts as an individual and as a member of the group.

Table 1 Frequency of social play bouts initiated by individual horses

Initiation	H1	H2	H3	H4	H5	H6	H7	H8
Sole	4	15	49	9	15	17	2	4
Group	0	0	19	2	3	15	2	5
Total	4	15	68	11	18	32	4	9
% of bouts	3.0	11.2	50.7	8.2	13.4	23.9	3.0	6.7

Conclusion Social play was observed in the established group young horses and all individuals were involved in its initiation. The majority of social play bouts were initiated by a single individual, however up to 6 individuals were involved in the initiation of a single play bout. Although all individuals were observed to initiate social play they were not equally represented with one individual involved in the initiation of more than half of the social play bouts observed. These results highlight that social play is a regular part of the behavioural repertoire of young horses, even when in an established group. Young horses kept in isolation will therefore be lacking the opportunity to express these behaviours that literature is increasingly demonstrating have real and adaptive benefits to the developing animal. Whilst further research in this area is required, there is sufficient weight of evidence to suppose that welfare may be reduced by preventing the expression of these behaviours.

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Evaluation of the performance of in-calf dairy heifers either housed or out-wintered on forage brassicas (stubble turnips)

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Introduction There is increased interest in out-wintering systems which have the potential to reduce costs and hence increase farm profitability. Out-wintering also offers the potential to increase herd size, within the constraints of Nitrate Vulnerable Zone (NVZ) restrictions without the substantial capital costs associated with waste storage. The majority of out-wintering research has focused on kale and its utilisation by dry dairy and suckler cows where relatively modest performance levels are required. In a comprehensive review on forage brassica for out-wintering stock (MDC 2007) it was stated that “despite the developments in New Zealand in the use of brassica based out-wintering systems for dry dairy cows, there seems to be no detailed studies on the use of these systems for growing (0.7-0.85kg daily live weight gain [DLWG]) dairy heifers”. The objective of this experiment was therefore to evaluate the performance of replacement dairy heifers either housed or out-wintered on forage brassicas (stubble turnips).

Materials and methods Twenty eight in-calf Holstein heifers weighing 478kg with a mean age of 21.8 months were either housed or out-wintered. The housed heifers were fed *ad libitum* grass silage (11.1 ME MJ/kg DM) and wholecrop (10.3 ME MJ/kg DM). They were initially fed 1.5kg/concentrates/day. This was replaced with 100g of minerals after 48 days. The out-wintered heifers on stubble turnips were initially offered *ad libitum* straw however this was replaced with haylage (10.6 ME MJ/kg DM) after 48 days. Mineral blocks (Rumenco Cattle Plus TABs) were offered for free access feeding. The electric fence was moved three times a week. This was implemented to reduce labour costs and it was not an objective to achieve 70-100% utilisation of the stubble turnips since it was considered that this could restrict dry matter intakes and hence DLWG on a ‘low cost system with extensive stocking’. Both groups of heifers were turned out onto grass on 20/3/08 and performance subsequently monitored until 1/7/08. The data was analysed using ANOVA.

Results The initial negative DLWG’s for the out-wintered heifers necessitated the change from feeding straw to haylage. Significant ($P<0.001$) compensatory growth was subsequently recorded. The housed heifers recorded a marked growth check following turnout ($P<0.001$) which therefore resulted in there being no significant differences in overall DLWG from start to finish. There were no significant differences in locomotion or dirtiness score and condition scores, except after 48 days when the housed heifers recorded a higher ($P<0.05$) score for the latter.

Table 1 Animal Performance – DLWG (kg)

	Housed	Out-wintered	s.e.d	Sig
Start - 48 days	0.77	-0.30	0.090	***
48 days - turnout	0.90	1.37	0.085	***
Start - turnout (120 days)	0.85	0.70	0.063	*
Turnout - 34 days	0.19	1.00	0.089	***
Turnout - finish (103d)	0.78	1.08	0.061	***
Start - finish	0.82	0.88	0.037	NS

Table 2 Animal Performance – Liveweight (kg)

	Housed	Out-wintered	s.e.d	Sig
Start (21/11/07)	476.6	476.4	11.45	NS
Turnout (20/03/08)	578.1	560.4	15.45	NS
Finish (1/07/08)	658.6	672.1	17.72	NS

Silage dry matter intakes of 10.2kg/d were recorded for the housed heifers. Estimated intakes of stubble turnips ranged from 37-51kg/h/d (4.2-5.8kg DM/h/d) plus 7.5kg/h/d (6.2kg DM) of haylage and 90g/h/d of mineral block for the out-wintered heifers. Utilisation of the stubble turnips was estimated at 38-44%. The majority of the stubble turnips left ungrazed were the roots with the heifers having a preference to eat the leaves. It was estimated that utilisation of the leaves was 90%. In this study 0.75ha provided sufficient stubble turnips for 1 heifer for 120 days.

Conclusions Target DLWG’s of 0.7-0.85kg to achieve 2 year calving can be achieved with out-wintering systems based on stubble turnips and haylage on a low cost system. Supplementary forage to stubble turnips must be provided from haylage/silage and not straw. Out-wintered heifers do not suffer a growth check when moved onto a grass sward in the spring. There were no welfare implications for the out-wintered heifers. Overall (winter and summer) variable costs per kg gain were reduced by 25% with out-wintering (58p v 77p/kg) with fixed and variable costs being reduced by 48% (0.78p v 150p/kg).

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Prediction of conception at 1st artificial insemination using a combination of energy balance indicators

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Introduction Declining reproductive performance is a major problem for the global dairy industry (Lucy 2001) whereas magnitude and duration of postpartum negative energy balance of dairy cows are considered as the main reasons (de Vries and Veerkamp 2000). Moreover, various energy balance indicators, such as body condition score (BCS) and plasma β -hydroxybutyrate (BHB) concentration, have been correlated with reduced reproductive performance (Pryce et al 2001, Taylor et al 2003, Patton et al 2007, Walsh et al 2007). Such information has been already used to adjust herd management practices in order to prevent negative effects on reproduction. Furthermore, the ability to predict reproductive performance of cows with reasonable accuracy would also be very useful to dairy farmers. In such case, important management decisions (e.g. length of voluntary waiting period, starting dates of synchronisation programs and price of semen used), could be made for each individual cow. The aim of this study was to investigate whether combining certain energy balance indicators would yield useful predictions of cow reproductive performance at 1st artificial insemination (AI).

Materials and methods Data from 262 first lactation Holstein cows were used. All cows were kept on the same commercial farm in Northern Greece and calved between 2005 and 2006. Weekly estimates of BCS and BHB were available for these cows as well as conception at 1st AI. In a preliminary analysis, the latter was regressed on separate weekly estimates of BCS and BHB revealing that BCS at weeks 2 and 6 (BCS₂ and BCS₆), and BHB at week 6 (BHB₆) had the largest individual effect on conception. These estimates, together with BCS loss between weeks 2 and 6 (BCS_{loss}) were considered for further analysis. Desirable energy balance indicator levels were defined as: BCS₂ $\geq$ 2.50, BCS₆ $\geq$ 2.25, BCS_{loss} $\leq$ 0.50 and BHB₆ $\leq$ 0.90 mmol/L; undesirable levels were BCS₂ $<$ 2.50, BCS₆ $<$ 2.25, BCS_{loss} $>$ 0.50 and BHB₆ $>$ 0.90 mmol/L. All four energy balance indicators were combined in pairs, threes and fours, each resulting in 4, 8, and 16 level combinations, respectively. All possible combinations of trait levels were then considered in relation to the corresponding cow conception rate. For example, conception of cows with high BCS₂ and low BHB₆ was compared to conception of cows with high BCS₂ and high BHB₆. Groups of cows were then formed consisting of combinations yielding similar conception rates. These groups were statistically compared to each other using relative risk (RR) and its 95% confidence interval (CI).

Results The combination of BCS₂, BCS_{loss} and BHB₆ turned out to be the most efficient at predicting conception rate. Cows with low BCS₂ and high BHB₆ (regardless of BCS_{loss}) as well as cows with high BCS₂, BCS_{loss} and BHB₆ (Group A), were the least fertile as 48 in 49 failed to conceive at 1st AI. For all remaining cows (Group B), 171 in 213 failed to conceive at 1st AI. The RR between the two groups was 1.22 (95% CI=1.13-1.32) meaning cows in Group A would be 22% more likely to fail to conceive than Group B cows. This difference was highly significant (exact Fisher's $P < 0.001$). When BCS₆ was also considered in Group B cows, those with low BCS₆ (subgroup B1) tended to have a lower conception rate at 1st AI (50 in 58 failed to conceive) than cows with high BCS₆ (subgroup B2; 121/155, RR=1.10, 95% CI=0.97-1.26, exact Fisher's $P = 0.10$). Most cows (88%) in Group B had a desirable BHB profile in week 6. The rest of them did not, but had a reasonable BCS in week 2 postpartum and lost minimum condition between weeks 2 and 6. Data from more cows are needed if a statistically significant difference between subgroups B1 and B2 is to be revealed. Cows with low BCS post-calving (week 2) and still being in negative energy balance (high BHB) in week 6 are indeed expected to have a low conception rate at 1st AI. The same proved to be true for cows that, despite a normal BCS after calving, lost significant condition and were still in negative energy balance in week 6 postpartum. Breeding these cows (Group A), whether following synchronization or not, should be delayed or lower price semen should be used. The latter could also be an option for cows of subgroup B1. However, genetic merit of these cows and their potential as replacement dams should also be considered. Cows of subgroup B2 (high BCS₂, high BCS₆, low BCS_{loss} and low BHB₆) are the most fertile of all and none of the above restrictions should be applied.

Conclusion Results of this study indicate that combinations of energy balance indicators could be effectively used to divide cows in three groups regarding their chances to conceive at 1st AI (low, moderate, high probability). This can help dairy farmers decide the length of voluntary waiting period, when to start synchronization programs and the price of semen used, for each individual cow. Similar studies, including larger number of both first and later parity cows are necessary to confirm these findings. Moreover, economic analysis of these management decisions should be performed.

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Effect of dietary energy content pre- and post-calving on production and blood metabolites of dairy cows during early lactation

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Introduction The increase in milk production potential of the modern high yielding dairy cow has resulted in excessive and prolonged negative energy balance (NEB) during early lactation. This predisposes the cow to an increased risk of metabolic disorders, poor fertility and subsequently increased culling rates. Historically, much emphasis has been placed on post partum nutrition in an attempt to suppress these ill effects. However, contemporary theories advocate improved dry cow nutrition to properly prepare the cow for energy demands of early lactation. Friggens *et al.* (2004) stated that priming the liver during the dry period would allow the cow to better deal with metabolic processes in the post partum period. In this experiment, dairy cows were offered different dietary energy levels pre- and post-calving in an attempt to quantify the effects on energy parameters during the same time periods.

Material and methods Forty Holstein heifers and forty Holstein cows (mean parity 3.2) were allocated to one of four treatments, based on a 2x2 factorial design: high or low energy density diet pre- and post calving. From day -80 until day -21 pre-calving, heifers on high and low pre-calving dietary treatments were offered high and low pasture allowances respectively. From day -21 until calving, heifers were housed and offered *ad lib.* or restricted diets (6 kg dry matter (DM)/day) depending on their respective treatments. The pre-calving treatment for cows commenced 100 days prior to predicted parturition date. All cows were housed and high and low energy diets were offered *ad lib.* From day 42 pre-calving, cows receiving the low energy diet were restricted to 6 kg DM complete diet/day, while the high energy pre-calving diet continued to be fed *ad lib.* Post calving, treatments were balanced for parity, body weight and date of calving. The concentrate: forage DM ratios of the high and low energy density diets post calving were 70:30 and 30:70 respectively, providing 12.5 and 11.7 MJ ME/kg DM. Consequently, there were four treatment groups; AH, AL, RH and RL.

Results Pre-calving, animals allocated a restricted low energy diet and an *ad lib.* high energy diet had significantly ($P<0.001$) different ME intakes; 68.0 and 95.2 MJ respectively ($P<0.001$; SED, 3.32). Pre-calving dietary treatment had a significant effect on body condition score at calving; on average animals receiving an *ad lib.* high energy diet and a restricted low energy diet had a body condition score (BCS) at calving of 2.70 and 2.49 respectively ($P<0.001$; SED, 0.061). There was no significant effect of pre-calving nutritional regime on plasma non-esterified fatty acids (NEFA) concentrations in the pre-partum period. Animals offered a restricted low energy diet and an *ad lib.* high energy diet had plasma NEFA concentrations of 0.41 and 0.38 (meq/l) respectively (SED, 0.037). The results presented in Table 1 illustrate pre- and post calving dietary influences on energetic parameters in the post partum period.

Table 1 Effect of dietary energy content pre- and post calving on post partum energetic parameters (0-100 days)

Pre-calving Post-calving	Parity	Ad lib. High	Ad lib. Low	Restricted High	Restricted Low	Pre	Post	Interaction	SED
Milk yield (kg/day)	1	30.0	24.2	27.8	26.0	ns	***	ns	1.52
	2+	36.0	31.9	36.0	30.3				
ME intake (MJ/day)	1	189	146	203	157	ns	***	ns	9.38
	2+	252	199	261	182				
TER (MJ/day)	1	206	178	193	192	ns	***	*	3.79
	2+	244	230	242	222				
CEB (MJ) ¹	1	-1519	-2204	2	-2106	***	***	***	115
	2+	-164	-2441	345	-2453				
Glucose (mmol/l)	1	3.54	3.51	3.66	3.54	*	**	ns	0.058
	2+	3.45	3.29	3.55	3.37				
NEFA (meq/l) ¹	1	0.432	0.451	0.366	0.404	*	ns	ns	0.038
	2+	0.339	0.422	0.282	0.351				

¹ NEFA, Non Esterified Fatty Acids; CEB, Cumulative Energy Balance; TER, Total Energy Requirement

Conclusions The results indicate that a restricted low energy pre-calving diet significantly reduced body reserve mobilisation post calving (indicated by plasma NEFA concentrations) resulting in a more rapid return to positive energy status. This concurs with Grum *et al.* (1996) who suggested that loss of body condition during the dry period reduced body reserve mobilisation post-calving, therefore maintaining liver function. In addition, a restricted low energy pre-calving diet significantly increased plasma glucose levels post-calving, which may also be indicative of improved liver function.

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An evaluation of the effect of nutritional strategy in early lactation on reproductive performance of Holstein-Friesian dairy cows

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Introduction Poor fertility is a major problem on dairy farms. On-farm studies indicate that pregnancy rates to first service have declined from approximately 55% between 1975 and 1982 to 39% between 1995 and 1998 (Royal *et al.*, 2000), illustrating a decline of approximately 1% per year during this period. The cause is multifactorial and influenced by many factors including genetics, management and nutrition. Previous studies have reported improved fertility through nutritional modification in early lactation (Gong *et al.*, 2002). The aim of this study was to evaluate the effect of nutritional strategy in early lactation on reproductive performance.

Materials and methods Ninety-six HF dairy cows were allocated to four dietary treatments. Multiparous animals (n=58) were balanced for milk yield in the previous lactation, parity (mean, 2.4), live weight and body condition score at calving. Primiparous animals (n=36) were balanced for live weight and body condition at calving. All diets were offered *ad libitum* as total mixed rations (TMR). The "Control" diet was formulated using Feed into Milk (Thomas, 2004) and contained an overall crude protein (CP) content of 175 g/kg DM. An Individual Cow treatment involved offering a basal TMR of 175 g CP/kg DM. Dietary protein content was adjusted on the basis of calculated weekly energy balance (EB), with protein reduced to 150 g/kg DM (if cows exceeded a predetermined negative EB) or increased to 200 g/kg DM (if cows exceeded an EB threshold). A third treatment (High/Low Starch) involved offering a high starch diet for 50 days postpartum (days 1-35 and 36-50 of 200 and 300 g starch/kg DM respectively). From day 51 postpartum to positive pregnancy diagnosis, animals were offered a low starch diet (100 g starch/kg DM) with additional protected fat (calcium salts of palm fatty acids) (37.5 g/kg DM). The fourth treatment (Low Protein) contained 150 g CP/kg DM, with an additional supplement of digestible methionine. Ovarian activity was assessed from milk samples taken at the morning milking on three occasions each week from parturition to confirmed pregnancy and analysed for progesterone using enzyme-linked immuno sorbent assays following the method of McCoy *et al.* (2006). Data were analysed using REML, linear and logistic regressions using Genstat.

Results Animals on the Low Protein treatment had a significantly lower ($P<0.05$) incidence of delayed ovulation type II. Incidence of atypical cycles was high in all dietary treatments (80%). Across all treatments, there were significant ($P<0.001$) positive correlations between the interval to commencement of luteal activity and peak milk yield, interval to peak milk yield and milk yield at time of energy balance nadir.

Table 1 Treatment effects on fertility parameters

	Control	Individual Cow	High/Low Starch	Low Protein	s.e.	Sig.
Commencement of luteal activity (d)	29.7	29.8	29.7	29.8	3.4	NS
Delayed ovulation type I (%) ¹	13.6	16.0	12.5	21.7	7.4	NS
Delayed ovulation type II (%) ¹	59.0 ^a	40.0 ^b	39.2 ^b	33.3 ^b	5.15	**
Persistent corpus luteum type I (%) ¹	13.6	32.0	33.3	21.7	8.7	NS
Persistent corpus luteum type II (%) ¹	20.5	30.0	28.4	20.9	4.5	NS
Animals with 1 or > atypical cycles (%)	82.1	86.8	73.7	79.9	7.13	NS
Animals with 2 or > atypical cycles (%)	36.6	52.0	45.8	21.7	9.7	NS
Days to conception from start of breeding	80.4	71.9	80.0	86.5	13.5	NS
Pregnant to 1 st insemination (%)	36.0	23.5	34.2	31.0	8.4	NS
Pregnant to 1 st + 2 nd inseminations (%)	59.0	65.8	54.1	58.8	9.0	NS
In calf rate after 100 days of breeding (%)	72.3	77.7	70.9	70.8	12.0	NS

¹Percentage of cycles that are atypical; ^{a,b} Means in the same row with different superscripts differ significantly, $P<0.01$.

Conclusions The occurrence of a delayed ovulation type II (42.9%) and persistent corpus luteum type II (25.0%) was higher than reported by McCoy *et al.* (2006) (13 and 11.3% respectively) and Royal *et al.* (2000) (10.6 and 16.8% respectively). The increase in persistent corpus lutea type II may reflect a high incidence of embryo loss in early pregnancy. The incidence of animals with one or more atypical progesterone profiles (80.6%) was higher than reported by McCoy *et al.* (2006) and Royal *et al.* (2000) (42% and 43.7% respectively). The high incidence of atypical progesterone profiles may overshadow any potential treatment effects on reproductive performance.

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The effect of growth rate on age at first calving, fertility and milk production during the first lactation of Holstein-Friesian heifers on UK dairy farms

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Introduction Rearing heifer replacements that reach first calving at an age and body weight (BW) to achieve their full lifetime potential, in terms of both yield and profitability, is a key factor in dairy enterprises. Rate of growth during the rearing period has a direct effect on age at first calving (AFC) (Heinrichs, 1993). It is widely accepted that the average daily gain (ADG) of Holstein-Friesian heifers should be approximately 0.7 kg/d before puberty, and 0.8 kg/d after. However, we have previously found extreme variability in the ADG of calves both within and between commercial dairy farms; this may in part be due to the very limited use of weight or height measurements on most farms. The aim of the present study was to determine the effect of growth rate during the first six months of life on AFC, and subsequent fertility and milk production during first lactation of Holstein-Friesian heifers on UK dairy farms.

Materials and methods A total of 17 dairy farms across southern England milking Holstein-Friesian cows were recruited during 2003-04, providing a range of management practices representative of those commonly encountered in the UK. For heifer calves recruited, BW was measured at one (28 ± 0.8 d) and six months (184 ± 0.8 d) of age, to calculate the ADG from one to six months. Heart girth and height at withers were measured at one to two wks before first calving. After calving, measures of fertility (AFC, days to commencement of luteal activity (CLA), days to conception (DTC), and services per conception (S/C)) and milk production (days in milk (DIM), milk per d, 305 d yield and total milk yield) were recorded. Animals with complete growth, fertility and milk production records ($n=283$) were subdivided on the basis of their pre-pubertal ADG; (i) <0.6 kg/d ($n=69$), (ii) $0.6-0.8$ kg/d ($n=91$) and (iii) >0.8 kg/d ($n=123$). One-way analysis of variance (ANOVA) and the post-hoc tukey test were used to compare the size, fertility and milk production traits between these groups. All data were tested for homogeneity of variance and log transformed if necessary. Chi-square analysis was used to compare the proportion of animals pregnant 200 d after calving between the three groups.

Results The ADG from one to six months was 0.76 ± 0.01 kg/d (range per calf 0.26 to 1.23 kg/d), and the mean AFC was 26.0 ± 0.2 months (range 21 to 40 months). Fertility and milk production traits during the first lactation according to ADG are presented in Table 1. Heifers with an ADG of <0.6 kg/d were smaller but on average three months older at first calving compared to those with an ADG of >0.8 kg/d ($P<0.001-0.01$). After calving, these smaller but older animals tended to have a longer interval to the CLA ($P<0.1$); heifers with the shortest interval to the CLA had an ADG of $0.6-0.8$ kg/d. There was no significant difference in the number of DTC or S/C between the three groups ($P>0.15$), but a larger proportion of animals with an ADG of $0.6-0.8$ and >0.8 kg/d were pregnant by 200 d ($P<0.05$). First lactation milk production was not significantly different between the three groups, although heifers with an ADG of >0.8 kg/d tended to produce less milk ($P<0.15$). An additional 19 heifers failed to conceive as a maiden heifer (<0.6 kg/d: 3%, $0.6-0.8$ kg/d: 4%, and >0.8 kg/d: 5%) and 24 during first lactation (<0.6 kg/d: 5%, $0.6-0.8$ kg/d: 8%, and >0.8 kg/d: 6%).

Table 1 Mean ($\pm$ SEM) size at first calving, fertility & milk production traits during first lactation of 283 heifers according to ADG; <0.6 kg/d, $0.6-0.8$ kg/d & >0.8 kg/d. +within rows a<b. NS=not significant $P>0.15$

Parameter		ADG (kg/d)			P-Value+
		<0.6	$0.6-0.8$	>0.8	
Size before calving	Girth (cm)	198 ± 1^a	203 ± 1^b	203 ± 0.6^b	0.001
	Height (cm)	137 ± 0.8^a	139 ± 0.7	141 ± 0.4^b	0.001
Fertility	AFC (months)	28 ± 0.5^b	26 ± 0.2	25 ± 0.3^a	0.01
	CLA (days)	34 ± 4^b	24 ± 2^a	26 ± 1	0.06
	DTC	148 ± 13	132 ± 9	123 ± 7	NS
	% pregnant at 200d	75%	85%	89%	0.04
	S/C	2.3 ± 0.3	2.5 ± 0.2	2.3 ± 0.1	NS
Milk	DIM	359 ± 13	353 ± 10	341 ± 7	NS
	Total milk (kg)	9857 ± 510	9980 ± 327	9059 ± 196	0.11
	Milk/d (kg/d)	27 ± 0.7	28 ± 0.6	27 ± 0.4	NS
	305 d yield (kg)	9231 ± 310	9122 ± 232	8633 ± 147	0.12

Conclusion An increased ADG during the first six months of life significantly reduced the AFC; after first calving a larger proportion of these animals were pregnant by 200 d. Increasing ADG, often through high planes of nutrition, presumably increases rearing costs; likewise a low ADG will lengthen the non-productive period. Therefore, we would suggest rearing heifers at a moderate growth rate of between 0.6 and 0.8 kg/d during the first six months of life. More effort should be made within the industry to ensure all heifers within a cohort reach this target.

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Key indicators and decision-making trees to analyse reproduction problems linked to environmental factors: data from a commercial dairy unit

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Introduction Reproduction performances in cattle regularly decreased with time. For example, the number of inseminations per pregnancy increased in dairy herds from 1.43 to 1.75 and the calving interval (CI) from 385 to 412 days in 10 years (Leroy *et al.*, 2006). Such a reduction is responsible for rising income losses for breeders. In Belgium, an increase of the age at first calving (AFC) by 3 months and of the CI by 20 days induced a gross margin reduction of about 500 € /cow/year.

Material and methods Two tools were developed with Association Wallonne de l'Elevage (AWE), a Region Wallonne supported company involved in cattle breeding and Corilus, a software company. The first tool is a computer program. The veterinarians of the AWE inseminator network use a PDA (personal digital assistant) directly related with the AWE data base. They define firstly with the farmer the expected reproduction performances (CI, AFC). Then, on each reproduction visit, they calculate, in the farm and on-line, key indicators (fertility and fecundity) to objectivise the real performances and compare them to the expected ones. The chosen key indicators are the projected CI or AFC, calculated with the days open (interval between calving and the pregnancy) of the entire herd, the conception rate at first insemination (CRFAI), the heat detection rate (HDR) and the rate of effective heat (REH). When the targets are not obtained, a methodology of diagnosis of environmental problems linked to reproduction is available. This is the second tool. Three decision-making trees (one for each reproduction problem: heat detection, infertility or abortion) organised in four levels allow the user to progress with logic and method to adjust the diagnosis.

Results The use of this methodology is summarised in the Table 1. Data are collected by different operators (scientists, farmers, veterinarians, feed traders etc.). After the reproduction visit and when the reproduction problems are defined, a meeting is organised to point causes. Many parameters are collected but only the most pertinent ones are summarised in the table.

Table 1 Summary of the most pertinent key indicators in a dairy farm with reproduction problems.

Parameters	Observations			
Description of the herd	Herd comprised 145 Holstein cattle of reproduction age with a quota of 850 000 litres. Cows and heifers kept on free stalls with sawdust.			
Key indicators of reproduction	Fecundity	Expected AFC: 26 months CI : 390 days Waiting period : 80 days	Projected AFC: 29 months CI: 426 days. Waiting period: 86 days	
	Fertility	CRFIA: 29% HDR: 72%	REH: 21% (goal: at least 25%)	
	Disease prevention	Vaccinations against IBR, BVD and worm prevention correctly carried out. Annual serology of <i>Fasciola Hepatica</i> was negative		
First level : Anamnesis	Frequent pathology	Frequency of about 10% of milk fever.		
	Nutrition	Ration calculated for production animals but not for heifers and dry cows. Rations not balanced for energy/protein amount.		
	Building	Stall for the production animals dark and insufficient space per animal. Heifer stall affected by the wind.		
	Body score	Very heterogeneous for both production and non-productive animals		
	Heifers	They had shaggy hair and subjected to constant wind		
First level: Clinical examination	Production cattle	Lameness and oedema of the hock frequently observed.		
	Second level: Further investigations	Milk	Average milk yield 25.2 kg/day. Energy balance (from fat/ protein ratio) in excess for 21% of cattle, increasing to 24% for repeat breeders. From milk urea data, 26% of cows had excess protein intake and 36% a deficiency.	
		Blood	The antigen test for blue tongue was positive. The mineral status was good.	
Third and fourth levels : possible causes -metabolic link to reproduction problem	Unbalanced intake for all animals could decrease hormones (prostaglandins, IGF-1etc.) and metabolites (glucose, fatty acids etc.) thus impairing ovocyte quality. Diet may cause high incidence of lameness and milk fever and affect reproduction performances (heat quality and detection. etc.)			
	Blue tongue: hyperthermia (embryo mortality and abortion), infected ovocytes.			
	Lack of space and light may induce poor heat detection and conception rate at first insemination.			

Conclusion The development of such tools allowed the field operators to objectivise, analyse and diagnose environmental factors accountable for reproduction failures. The reproduction performances are very sensible to the environmental factors and a decrease of these performances can lead to economical losses. So, this approach seems to be relevant, especially because it includes preventive and curative managements. The data collection is carried on by a large team which can adjust, question itself and improve this methodology with the economical interest of the farmer as an ultimate goal.

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Ovarian status, uterine pathology and physical condition of anoestrus and culled buffaloes in Chitwan, Nepal

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Introduction Buffaloes are the major production animals in most of the South Asian countries including Nepal. The buffalo, however, is a sluggish breeder and is beset with various constraints which adversely influence its fertility; such as, problems of silent heat coupled with poor expression of oestrus, irregular oestrus cycles, anoestrus, prolonged days open and low conception rates (Madan and Prakash, 2006). Among them, anoestrus is the most commonly encountered condition leading to reproductive failure and culling in buffaloes. However, the ovarian status of such buffaloes is poorly understood. The present study aims to investigate the ovarian status, uterine pathology and physical condition of anoestrus and culled buffaloes in Chitwan, Nepal. The buffaloes in Nepal are generally culled if they fail to conceive for long time or the production is seized.

Materials and methods The present study was conducted on Murrah type buffaloes in Chitwan, Nepal. In experiment 1, buffaloes (n=124, parity=1.86±0.12) with the history of prolonged anoestrus were included for study. These animals were reared by the small holders under farmer's management condition and also in the Institute of Agriculture and Animal Science (IAAS) Livestock Farm, Rampur. The ovaries of buffaloes (n=79) were examined manually per rectum or by using transrectal ultrasonography (HV1500, Honda Electronics, Japan) to identify the ovarian status on the basis of the presence or absence of functional structures such as the corpus luteum (CL) and the follicles (≥7.0mm) in the paired ovaries and classified them as buffaloes having functional ovaries (FO) or non-functional ovaries (NFO), respectively. The uteri of these animals were examined to diagnose the inflammatory conditions such as endometritis and for uterine discharge. The body condition score (BCS) of buffaloes (n=53) was recorded as described by Ferguson et al. (1994) for the cattle. The faecal material of buffaloes (n=73) was also examined as routine test for endoparasites. In experiment 2, the paired ovaries and uteri of culled buffaloes (n=37) were collected from the local slaughterhouse for morphometric study. The specific reason of culling of these buffaloes was not known. The functional structures in the ovaries such as the CL and follicles (≥7.0mm), if present, were observed and measured grossly and recorded as buffaloes having FO, and if not present, as NFO. The uteri of these animals were also examined for endometritis and uterine exudates. Data were pooled and analyzed by using JMP 5.0.1 (SAS, Japan) software. The BCS between buffaloes having FO and NFO were compared by student's *t* test.

Results The faecal examination of anoestrus buffaloes revealed 82.19% (60/73) positive cases for endoparasites. The ovarian status, uterine pathology and physical condition of anoestrus buffaloes and the ovarian status and uterine pathology of culled buffaloes are summarised in Table 1.

Table 1 Ovarian status, uterine pathology and physical condition of anoestrus buffaloes and the ovarian status and uterine pathology of culled buffaloes

Animals (n)	Ovarian status (%)	Endometritis (%)	BCS (n=53)
Anoestrus buffaloes (79)			
FO (52)	FO (65.82)	3.79	2.76±0.06 ^a
NFO (27)	NFO (34.18)	1.26	2.29±0.04 ^b
Culled buffaloes (37)			
FO (23)	FO (62.16)	0.00	NR
NFO (14)	NFO (37.84)	5.41	NR

FO: functional ovaries, NFO: non-functional ovaries, BCS: body condition score, NR: not recorded

Mean values of BCS with different superscripts were significantly different, *P*<0.01.

Conclusion The results of this study showing an association of anoestrus with endoparasitic infestation, suggest that in Nepal, endoparasitic infestation could be the major cause of anoestrus in buffaloes. The uterine pathology was not a major problem in anoestrus as well as in culled buffaloes. BCS is indicative of the ovarian function. The ovarian status of majority of anoestrus and culled buffaloes revealed the presence of FO. We suggest that the challenge of buffalo breeding in Nepal can be managed by applying the regular anthelmintic programs and improving nutritional management. The presence of FO in the majority of anoestrus and culled buffaloes may indicate the utilization of ovulation induction and fixed time insemination protocols to overcome the problems of buffalo breeding and prevent economic loss due to culling.

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Changes in the bacterial populations in the equine hindgut following the addition of inulin to the diet

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Introduction Laminitis in the horse is associated with the over consumption of rapidly fermentable carbohydrate, in the form of simple sugars, fructans and/or starch. The fermentation of excessive carbohydrate in the hind-gut can result in the production of lactic acid and other toxins which in turn may act as "laminitis trigger factors." (Crawford et al., 2007). However, whilst it is known that susceptibility to laminitis varies within the equine population, little is known about the factors governing this variation. We have previously shown that 16S *rRNA* t-RFLP profile from bacteria extracted from equine faeces clustered weakly into two clades representing normal versus laminitis-prone ponies suggesting a difference in gut microflora between the two groups (Newbold et al., 2008). Here we have extended this observation using real time PCR to investigate changes in the number of *streptococci* and *lactobacilli* in the faeces of normal and laminitis-prone ponies following fructan administration.

Materials and methods Five normal and 6 laminitis-prone, native-breed ponies were acclimated to a basal hay diet before being fed a diet of inulin (3 g/kg of BW per day) hay plus chopped dried grass. No clinical problems occurred. Fresh faecal samples were collected at -4, -2, +2 and +5 days after diet change. DNA was extracted using the QIAamp® DNA stool mini kit (Qiagen Ltd, Crawley, West Sussex, UK). The quantification of 16S *rDNA* in the samples was performed by real time PCR on an Opticon system (GRI Ltd, Essex, Braintree, UK) as described previously (Duval et al., 2007). *Streptococci* were determined using 5' ATG TTA GAT GCT TGA AAG (AG)AG 3' as the forward primer and 5' CGC C(AT)T GGT GAG CC(GT) TTA 3' as a reverse primer, *lactobacilli* were determined using 5'TGC CTA ATA CAT GCA AGT CGA 3' and 5'GTT TGG GCC GTG TCT CAG T 3' respectively. Data are expressed relative to quantification of the total bacterial population quantified using the primers described by Maeda et al (2003)

Figure 1 Relative numbers of *lactobacilli*

Figure 2 Relative numbers of *streptococci*

Results There was no clear effect of either inulin addition or susceptibility to laminitis on the numbers of *lactobacilli* that could be recovered from the faecal samples (Figure 1), however there was a dramatic increase in *streptococci* numbers recovered from the faeces of laminitis-prone ponies, but not non-laminitis prone ponies, following the addition of inulin (Figure 2).

Conclusions These results add further evidence that the bacterial population of ponies responds differently to the addition of inulin to the diet in laminitic and non laminitis prone animals. A clearer understanding of these differences may in time allow us to design strategies to avoid the changes in bacterial population in the hindgut of the horse fed diets high in rapidly fermentable carbohydrate that in time result in laminitis.

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The effects of digestive enhancer supplements on digestibility in leisure horses

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Introduction Certain commercial horse feed supplements based on active yeast and its derivatives, and have been shown to improve digestion of feed (Medina *et al*, 2002), although this data is very limited and is mostly derived from studies in other species, such as pigs or cattle. Yeast-derived compounds are known to improve digestibility via different mechanisms, depending on their composition. Live yeasts interact at a gut level by removing any oxygen present, which can unbalance the fermentative microflora by promoting aerobic bacterial populations. This can lead to an increase in non-fermentative or pathogenic species, which increases the potential for diarrhoea or other digestive upsets. Yeast cell wall material (mannan-oligosaccharides) have been shown to bind pathogenic bacteria in the digestive tract, inhibiting their ability to reproduce, thereby stabilising the microflora and optimising fermentative capacity for fibre digestion, promoting animal growth and efficiency (Rosen, 2005). As horses rely on anaerobic fermentation to liberate energy from their natural high fibre diets, a stable and appropriate microflora is essential. The aim of the experiment was to investigate the benefits of feeding commercial digestive enhancers in horses with a history of gastric problems.

Materials and methods 24 adult horses, used for leisure riding, were used in a trial conducted at Massey University Equine Nutrition Facility in New Zealand. The horses used were selected based on a history of digestive problems which was verified by previous veterinary history and was characterised by problems with maintaining bodyweight, persistent diarrhoea and recurrent mild colic. The horses had all previously been grass fed only, with no previous exposure to digestive enhancers. Each animal was individually housed in a 4m x 3.6m outdoor pen, bedded on wood chip, with *ad lib* access to water. All horses had freedom to express natural behaviour and were allowed a supervised period of exercise each day, according to Massey University Animal Ethics Protocols. Hay (all grown in the same location field under identical management regime) and a freshly-made fibre-based balanced compound feed formulated to meet NRC requirements (Camtech Nutrition Ltd, Hamilton NZ) was supplied on an individual basis, according to body weight and condition score from d1 to d21. During the last week of the trial, 3 kg of feed (DE 18.2 MJ/kg DM) and 10 kg hay (16.7 MJ/kg DM) was fed to each horse. Any refusals were monitored and recorded. A total faecal collection was made in the last three days of the trial period digestibility (forage digestion assayed by AIA; Miraglia *et al*, 1999). Intake was recorded on a daily basis with any refusals of hay or feed monitored and recorded. All horses were condition scored (1-5 scale where 1=thin and 5=obese), weigh taped and assessed for faecal quality (1=diarrhoea, 5=firm pellets) at day 1, 14 and 28. Two treatments; a control (unsupplemented diet) or a digestive supplement (Yea-Sacc¹⁰²⁶ + Bio-MosTM at 10 g/h/d of each supplement) were fed in a randomised block design. Data were analysed by the GLM procedure of UNISTAT (Unistat Ltd, London, UK).

Results and conclusions Although horses on the control diet had a significantly lower faecal dry matter at the start of the trial, dry matter was normalised in the digestive supplement diet by day 28. Horses with sub-clinical gastric disorders fed the digestive supplement diet had improved faecal scores, which were evident even at 14 days of exposure, resulting in a significant change in faecal quality ($p < 0.05$). Faecal energy output, on a per gram of faecal matter basis, was reduced by 8.5%, indicating better energy utilisation in horses fed the digestive supplement for 14 days. After 28 days, horses fed the digestive supplement significantly improved their faecal scores compared to the control. Faecal output (on a dry matter basis) was 0.44 kg less in the horses fed the digestive supplement. Modifying the confidence limits according to other work with limited animal numbers ($P_{in/out} = 0.20$; after Rosen, 2005) indicated trends in higher feed intakes, lower faecal output, larger change in faecal pH and a 5.5% higher digestibility in the horses receiving the digestibility supplement.

Parameter (n=24)	Control	Yea-Sacc ¹⁰²⁶ + Bio-Mos TM	P value
Daily intake (kg)	9.44	9.76	0.158
Daily faecal output (kg DM basis)	4.19	3.75	0.172
Change in body condition score (BCS)	-0.25	-0.45	0.348
Weight change (kg)	4.1	5.7	0.951
Change in faecal score	+0.21 ^b	-0.50 ^a	0.034
Change in faecal pH	-0.13	-0.32	0.152
Forage digestibility (%)	46.5	52.0	0.153

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Linseed oil or sunflower oil as supplements in horse compound feedstuffs : effects on plasma insulin and on plasma triglycerides fatty acids

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Introduction Sport horses tend to be offered diets high in concentrate during trainings and races. These concentrates are mainly made of cereals, which are high in starch. Owing to this high level of starch, associated disturbances, such as gastrointestinal disorders or laminitis can be observed. Fat has been suggested as an alternative compound to starch. Oil can increase the energy content of the diet and also supplies essential fatty acids. Linseed oil, incorporated into equine diets has been shown to reduce the extent of the plasma insulin profile and the arachidonic acid (C20:4 n-6) content in the plasma, while it increased the alpha linolenic acid (C18:3 n-3) content (Istasse *et al*, 2007). The aim of the present trial was to compare linseed oil high in C18:3 n-3 with sunflower oil high in linoleic acid (C18:2 n-6), when incorporated in a compound feedstuff.

Material and methods Eight adult horses trained four days a week were used in 3 3X3 latin square designs, one of them being incomplete. Each period lasted for 2 months. They were offered a diet based on 50% grass hay and 50% compound feedstuff made of 47.5% whole spelt, 47.5% rolled barley 3% molasses and 1% mineral mixture in the control diet. Eight percents of barley was substituted by 8% of linseed oil or by 8% of sunflower oil in the 2 other diets. Blood samples were obtained every 20 minutes over a 8 hours period. Plasma insulin was measured every hour. Individual fatty acids were determined in the plasma triglycerides before feeding and 2 hours after. The statistical models included the effects of diets, latin squares and periods.

Results The fat content was low in the control concentrate at 15.4 g/kg DM. This rose to 94.1 g/kg DM when the oils were included. The oil intake was 0.278 kg/d. The horses remained healthy over the 6 months of the experiment. There were no evident refusals during the trial. Plasma insulin concentration rose after the meal to reach a plateau within 2 hours. The extent of the increase and of the plateau were reduced when both oils were added (Figure 1). Significant differences were observed between diets on most of the fatty acids in the plasma triglycerides. The effects were similar on both sampling times so that averages are given in table 1. Linseed oil increased the content of C18:3 n-3 (6.32 vs 2.07 mg/100ml, $P < 0.001$) and of C18:2 n-6 (78.8 vs 58.1 mg/100ml, $P < 0.10$). When sunflower oil was offered, the corresponding contents were 1.34 and 75.2 mg/100ml. The C20:4 n-6 content was reduced ($p < 0.001$) both with linseed oil (1.06 mg/100 ml) and with sunflower oil (0.91 mg / 100 ml) as compared with 1.15mg/100ml on the control diet.

Figure 1 Changes in plasma insulin in horses offered either a control concentrate or a concentrate supplemented with 8% of linseed oil or sunflower oil.

Table 1 Fatty acids profile in the plasma triglycerides fraction

mg/dl	Control	Sunflower oil	Linseed oil	SEM	P>F
C16:0	17.2	17.4	16.7	1.693	0.67
C18:0	18.1	23.1	24.4	1.311	0.001
C18:1 n9/7	15.1	12.9	12.6	1.977	0.05
C18:2 n6	58.1	75.2	78.8	18.72	0.10
C18:3 n3	2.07	1.34	6.32	1.896	0.001
C20:4 n6	1.15	0.91	1.06	0.095	0.001

Conclusion The reduced responses in insulin associated to the supplementation with linseed oil or sunflower oil suggest that these oils may be fed to reduce the negative effects of a high concentrate diet in the developmental diseases of the young horse intensively trained. Similarly, the reduction in the plasma C20:4 n-6 concentration can induce less proinflammatory reactions.

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Equine limb and oral fibroblasts in *in vitro* models of cell proliferation and wound contraction

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Introduction Wound healing in horses is particularly problematic compared to other species and limb wounds often exhibit complications such as exuberant granulation tissue, poor wound contraction and unsightly scars. In comparison, oral wounds heal without scar formation. Therefore, *in vitro* experiments were conducted to investigate the differences between equine oral and limb fibroblasts in terms of proliferation and their ability to contract a collagen matrix, a commonly used *in vitro* model of wound contraction.

Materials and methods Two clonal primary fibroblast cell lines were established from slaughterhouse material; one from tissue harvested from the buccal mucosa (oral), and one from tissue harvested from the dorsal aspect of the third metacarpal (limb) of a horse. In Experiment 1, the effect of 2% foetal bovine serum (FBS) plus either 10 ng/ml platelet derived growth factor (PDGF) or 1 ng/ml transforming growth factor (TGF) β 1 on the proliferation of the two cell lines was investigated. Controls contained 2% FBS. Cells were seeded in a 24-well plate, and after 5 days the number of cells was counted using a haemocytometer. Cells were also stained with propidium iodide and examined using flow cytometry to determine differences in viability. The experiment was replicated three times, each from a separate batch of cells and each replicate contained triplicate measures of each treatment. In Experiment 2, floating fibroblast populated collagen matrices (FPCM) containing 1.60 mg/ml collagen and 8.3×10^4 cells/ml were made for both cell lines. Treatments and controls were identical to Experiment 1. The surface area of the FPCM was determined immediately after it was made and then after 5 days using a digital scanner. The extent of the contraction of the FPCM was expressed as a percentage of the original area. Replication was as for Experiment 1. Results from both experiments were analysed by ANOVA followed by a least significant difference test.

Results Table 1 shows the cell counts of the two cell lines as a result of the different treatments. Oral fibroblast proliferation was greater than that of the limb cells for the control and PDGF treatments. PDGF stimulated proliferation compared to the control treatment for both cell lines. TGF β 1 did not affect the proliferation of limb fibroblasts relative to the control ($P > 0.05$) but it inhibited proliferation of the oral fibroblasts relative to the control. Oral cells treated with TGF β 1 also had decreased viability when tested with propidium iodide ($P < 0.001$; results not shown).

Table 1 Effect of PDGF or TGF β 1 on proliferation of limb and oral fibroblasts (Experiment 1)

	Limb Fibroblasts			Oral Fibroblasts			SED
	Control	PDGF	TGF β 1	Control	PDGF	TGF β 1	
Number of cells	10694 ^d	26542 ^b	11278 ^d	14875 ^c	37186 ^a	7188 ^e	1792

Means with different superscripts are significantly different ($P < 0.001$)

Table 2 shows the percentage contraction of the FPCM generated by the two cell lines as a result of the different treatments. Oral cells contracted FPCM significantly more than limb cells under all treatments, with PDGF stimulating an increase in contraction compared to both the control and TGF β 1. Both PDGF and TGF β 1 stimulated increased contraction of FPCM containing limb fibroblasts compared to the control.

Table 2 Effect of PDGF or TGF β 1 on % contraction of FPCM containing limb or oral fibroblasts (Experiment 2)

	Limb Fibroblasts			Oral Fibroblasts			SED
	Control	PDGF	TGF β 1	Control	PDGF	TGF β 1	
% contraction of FPCM	96.8 ^a	84.1 ^c	91.9 ^b	62.6 ^d	56.5 ^e	61.7 ^d	2.10

Means with different superscripts are significantly different ($P < 0.001$)

Conclusions Oral fibroblasts proliferate and contract FPCM to a greater extent than limb fibroblasts, and this may partially account for the faster healing of oral wounds. In both experiments, the two cell lines responded in a similar manner to PDGF, but the response to TGF β 1 varied. TGF β 1 is implicated in the formation of exuberant granulation tissue, which is characterised by uncontrolled fibroblast proliferation and their production of collagen (Wilmink and van Weeren, 2005). TGF β 1 did not affect proliferation of limb fibroblasts, suggesting that an abundance of TGF β 1 in limb wounds may permit proliferation, whereas TGF β 1 in oral wounds may switch off proliferation by decreasing cell viability. TGF β 1 also increased contraction in limb fibroblasts, but had no effect on oral cells. However, it is unlikely that this would have a noticeable effect *in vivo* due to the strong fixation of the skin in limbs and the minimal degree of contraction stimulated here by TGF β 1.

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Quantification of greenhouse gas emissions from a beef feedlot system in south-east Australia during summer conditions

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Introduction Feedlot management systems for beef cattle are becoming a more common practice in Australia reflecting opportunities to ensure quality of product whilst maintaining cost efficiencies within production. However, feedlot systems have been identified as point sources of greenhouse gases emissions (GGE: methane, nitrous oxide and the indirect greenhouse gas ammonia). It has been estimated that feedlot systems contribute 3.5% of total direct methane emissions (Alford et al. 2006), and 30% of total emissions from livestock wastes. Furthermore, approximately 1% of total N₂O emissions from agriculture are attributed to livestock. This paper reports methane, N₂O and NH₃ emissions from an Australian feedlot system managed under summer climatic conditions. It compares actual measured emissions with estimated from three recognised models used by national governments to estimate total GGE per annum from livestock agriculture.

Materials and methods The feedlot was in NW Victoria (36.6°S, 143.4°E) and was representative of typical operation in SE Australia. The feedlot capacity was 12,800 cattle (mixed genotype: European – Angus X or Hereford X) reared from approximately 200 kg to 600 kg (mean live weight 470 kg; range 290 to 620 kg) depending on market requirements. The rations offered comprised barley and wheat grain (0.48), grass silage (0.27), grass hay (0.05), whole cotton seed (0.05), bread waste (0.08), with additions of molasses, vegetable oil supplements (0.07). Open path tuneable diode lasers (Boreal, Laser Inc., Alberta, Canada) and FTIR mass spectroscopy (University of Wollongong, NSW, Australia) were used to measure concentrations of CH₄, N₂O and NH₃. A three-dimensional sonic anemometer coupled with a high speed data logger (Campbell Scientific, Utah, USA) measured wind and turbulence statistics. Emissions (flux) were generated from concentrations and microclimatic conditions using backward Lagrangian stochastic model (bLs) (WindTrax software (Thunder beach scientific, Canada; Flesch et al. 2007). The feed and animal performance data collected from the feedlot inventories were used to estimate enteric methane using IPCC (1996) Tier II (3% GEI), Blaxter and Clapperton (B&C; 1965) and Moe and Tyrrell (M&T; 1979). CH₄ emissions from manure were estimated using volatile solids and a methane conversion factor of 1.5% (temperate region). Emissions of N₂O and NH₃ from manure were estimated using emission factors of 0.02 and 0.3 of manure nitrogen respectively. Measured total system N₂O was adjusted for direct (animal) emissions. CO₂-E was calculated using a value of 21 for CH₄ and 310 for N₂O.

Results The emissions measured from the feedlot were comparable to calorimetric studies with beef cattle in North America and Canada (Moe and Tyrrell, 1979).

Table 1 Greenhouse gas emissions (measured and calculated) from an Australian feedlot system in summer.

	CH ₄ g/head/day	Manure CH ₄ g/head/day	N ₂ O g/head/day	NH ₃ g/head/day	CO ₂ -E kg/day	N ₂ O % CO ₂ -E
Field Observation	185.4 ±74.2	-	8.2 ±8.1	111.5 ±76.9	6.4	40
Estimates	-	3.4 ±0.002	5.8 ±1.7	55.5 ±16.1	-	-
Tier II	93.1 ±25.2	-	5.9 ±0.9 ^a	-	3.8	47
B&C	273.9 ±71.6	-	^a	-	7.6	24
M&T	185.9 ±35.6	-	^a	-	5.8	31

^a estimate assumed to be identical to Tier II for comparison

Ammonia emissions were comparable to studies in North America and Canada (Flesch et al., 2007). The current Australian methodology underestimates emissions of N₂O and NH₃ from manure and the contribution of N₂O to total CO₂-E.

Conclusions Methane emissions from Australian beef feedlots were similar to those reported for high grain feedlot diets in Canada and North America. The equation current used in the Australian methodology, M&T, provides the best estimate of feedlot methane production. Nitrous oxide contributes up to 40% of the CO₂-E emitted from the feedlot; manure management strategies may be effective at reducing total feedlot emissions.

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Decrease in methane emissions in dairy cows with increase in dietary linseed content

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Introduction Because ruminants make a significant contribution to greenhouse gas emissions, attempts are being made by dietary manipulation, to mitigate methane emissions without altering animal performance. Dietary fat supply may be a promising solution (Martin *et al.*, 2008a). A first experiment has shown that increasing extruded linseed supply in a hay-based diet resulted in a decrease in methane production (Martin *et al.*, 2007). A second experiment has been carried out using a maize silage-based diet and the same linseed supply. The present abstract summarises the results of both experiments.

Materials and methods Two successive experiments have been carried out in dairy cows according to Latin square designs.

In each experiment, four lactating Holstein dairy cows (initial body weight of 627 kg and 668 kg for experiments 1 and 2, respectively) were randomly assigned to four dietary treatments in 4 x 4 Latin square designs. Diets were control diets (experiment 1: 50% natural grassland hay and 50% concentrates; experiment 2: 60% maize silage and 40% concentrates, both on a dry matter (DM) basis) and diets supplemented with 5% (EL5), 10% (EL10), or 15% (EL15) of extruded linseeds, corresponding to an expected oil supplementation of 2, 4, and 6% of dietary DM, respectively. Cows had *ad libitum* access to the forage (10% refusals). The desired forage:concentrate ratio was maintained by daily adjustment of offered amounts of forage and concentrate, depending on the composition of the refusals of the previous day. Each experimental period lasted four weeks, with measurements occurring during the last week of each period. Milk yield was determined on 5 days, organic matter (OM) digestibility was measured by total faeces collection on 6 days and individual CH₄ productions on 4 days using the sulphur hexafluoride (SF₆) tracer technique as described by Martin *et al.* (2008b).

Data were analysed separately for each experiment as 4 x 4 Latin squares using the MIXED procedure of SAS. The statistical model included cow, period, treatment and residual error. Fixed effects included period and treatment. Cow was the random effect. Overall differences between treatment means were declared significant at $P < 0.05$.

Results For each experiment, no significant difference among diets was shown for DM intake (mean and SE: 20.0 and 0.70 kg/d for experiment 1; 18.0 and 1.08 kg/d for experiment 2) and for milk yield (mean and SE: 26.6 and 1.68 kg/d for experiment 1; 24.1 and 2.63 kg/d for experiment 2). Linseed supply significantly decreased methane emission in both experiments (Figure 1). The decrease was significant for EL10 and EL15 with hay diet, for EL15 with maize silage diet. The extent of the decrease was 15, 19 and 40% in experiment 1, and 6, 13 and 42% in experiment 2 for a supply of 5, 10 and 15% of extruded linseeds, respectively. These variations cannot be explained by changes in OM digestibility, which did not vary among diets (mean and SE: 71.5 and 0.84% for experiment 1; 67.4 and 1.91% for experiment 2).

Figure 1 Effect of dietary linseed content on methane emission in dairy cows receiving diets based on hay (experiment 1) or maize silage (experiment 2). Treatments with different superscripts differ ($P < 0.05$): a, b, c: experiment 1; x, y: experiment 2.

Conclusion These experiments show that a dietary supply of extruded linseeds decreases methane production both with hay and maize silage diets, without altering milk yield. The extent of the decrease depends on the dietary linseed content. This feeding regime is one of the most efficient yet studied to mitigate methane emissions, but it needs to be confirmed with a larger number of animals and by long-term experiments.

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Cost effectiveness of abating greenhouse gases from UK livestock systems

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Introduction Mitigating, or abating, greenhouse gases (GHG) from livestock systems can play a vital role in providing solutions to the UK's climate change obligations. The main abatement options from the livestock sector, independent of grazing/pasture management, are through the efficiencies with which ruminant animals utilise their diet and manure management. Options include breed selection, through manipulation of dietary regimes or manure storage strategies. Attempts to abate GHG emissions should first target those abatement options that are cost efficient and this study estimates the cost effectiveness of selected options for the UK livestock sector.

Materials and methods A literature review highlighted an array of abatement options for livestock falling into two broad categories, options that focus on animal management options and options that focus on manure management, for example on-farm anaerobic digestion (OFAD). The options were shortlisted based on their likely uptake and feasibility. Information on the abatement potential of each option for reducing GHG emissions were taken from a range of sources, including publications, reports and expert opinion. Other information collated or derived for each option included the applicability (percentage of animals to which the abatement options could be applied), the effect on productivity and/or the effect on feed intake. Other input data (e.g., animal numbers, livestock system parameters) were also collated. The cost of implementing each option was estimated using the annual cost of administering the abatement option per treated animal and multiplied by the number of animals treated. Quota was assumed for dairy measures, such that if a measure had a favourable effect on production, animal numbers and associated running costs would be reduced as fewer cows would be required to meet quota. Each of the abatement potentials and their cost-effectiveness was first studied on a stand alone basis over time, with results for 2022 presented herein. However, it is unlikely that all measures studied will work effectively together. Interactions between livestock measures were assumed to be either 0 or 1, such that 0 meant that the pair wise combination of measures could not be applied simultaneously and 1 meant that measures could be applied simultaneously and the effects could be additive. Considering interactions resulted in a much shorter list of options than the original, stand alone list.

Results The results (Table 1) show that a range of options, both animal and manure management options, have a high potential for the abatement of GHG from livestock systems. The cumulative effect of these measures show that an annual abatement of approximately 2.68 MtCO₂eq could be achieved in the livestock sector at a cost of ≤£100/t by 2022. This represents around 5% of the 2005 UK agricultural GHG emissions (44.7 MtCO₂eq, excluding land use change). It should be noted options are not currently feasible in the UK (e.g. the use of ionophores prohibited) but may become an option in the future. Some of the measures proved to be highly cost-effective such that adopting this measure would result in a cost saving to the farming system. For example, the use of recording and genetic selection tools in beef results in improvements in the systems that utilise these tools. However these tools are not used as widely as in other

livestock sectors. Improving the uptake of such tools and increasing the efficiency of production in some beef systems will have a large impact on overall GHG emissions but will also have an impact on the overall farm profit and sustainability and therefore be highly cost-effective. The results showed that OFAD tended to be only effective in larger scale farms.

Conclusions The results show there are a range of cost-effective options (negative or low cost) for the UK livestock sector that will abate GHGs. These options also result in a saving to the farm business. These results can be used to guide policy makers on options available and the feasibility of delivering cost effective GHG abatement for the UK livestock sector.

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Table 1 List of livestock GHG abatement measures the total volume of CO₂ eq GHG abated, and the cost effectiveness of that option for 2022.

Measure	Sector	Vol abated (kt CO ₂ eq)	Cost eff. (£/t CO ₂ eq)
Ionophores	Beef	347.38	-1,747.79
Wider uptake of genetic improvement	Beef	46.32	-3,602.93
Ionophores	Dairy	377.36	-0.07
Increase prop ⁿ maize silage in diet	Dairy	739.66	-48.59
Genetic improvement based on current breeding goal	Dairy	346.26	-0.04
Genetic improvement based on broader breeding goal (fitness traits)	Dairy	95.98	-262.63
OFAD (large farms)	Beef	47.77	0.96
OFAD (large farms)	Pigs	97.79	2.52
Central anaerobic digestion	Poultry	16.06	4.69
OFAD (medium farms)	Pigs	250.81	7.96
OFAD (large farms)	Dairy	219.34	11.43
OFAD (medium farms)	Beef	50.77	16.96
OFAD (medium farms)	Dairy	44.12	24.10
Bovine somatotrophin	Dairy	132.31	224.10
Use of offspring of "transgenically" improved animals	Dairy	504.29	1,691.28
Increased concentrate in diet	Beef	80.96	2,704.54

Using values economics to add mitigation of greenhouse gases to dairy selection tools

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Introduction The economic appraisal of greenhouse gases (GHG) emissions is complex. The shadow price of carbon (SPC) is derived from the best estimate of the present value of damages associated with a tonne of GHG emission in carbon dioxide equivalents (CO₂ eq). The SPC rises with time, reflecting the increasing marginal damage of a tonne of GHG when added to a growing stock of atmospheric GHGs. There are many possible technical mitigation options for livestock systems, one of which includes harnessing selection tools. The study of Stott et al. (2005) describes how relative economic values (REVs) are calculated for traits included in the UK dairy profit index (£PLI) using dynamic programming tools to model a whole farm system. The REV for each trait is calculated by examining the consequence of a unit change in a trait of interest on net farm revenue, while keeping all other traits in the index fixed. The SPC provides a useful mechanism of considering the costs of GHG emissions in an economic index framework, such as £PLI. This study outlines methods for incorporating the environmental value of emissions mitigation into breeding goals.

Materials and methods The model parameters used by Stott et al., 2005 and IPCC Tier II methodologies (IPCC, 2006) were used to model the methane, CH₄ (enteric fermentation and manure storage), and nitrous oxide N₂O (manure storage) emissions from the whole farm system (young stock and milking herd) used to calculate REVs for £PLI. The N₂O emissions due to nitrogen excretion when cows are grazing were also included. The shadow cost to the dairy herd of GHG emissions was calculated by multiplying the current value of the SPC (2008 value = £26.50/t CO₂ eq) by the total GHG emissions from the system. The REV considering the SPC for milk yield was then calculated by increasing milk yield in percentage units while holding all other traits fixed. The impact of improving milk yield on GHG emissions and therefore the overall shadow cost of emissions to the farm is that fewer cows and followers are required to maintain a fixed herd output.

Results Figure 1 shows that the largest proportion of GHG emissions from the defined dairy system is due to enteric fermentation in the milking herd, with over 59% of the total GHG emissions. The young stock contributed over 20% of the total GHG emissions, which included CH₄ from enteric fermentation and CH₄ and N₂O from manure storage. Overall, the dairy system produced approximately 791 t CO₂ eq per annum. This equated to annual shadow cost to the dairy herd of £20 957 per annum or 1.95 p per kg milk produced. Improving milk yield by 1% decreased the GHG emissions from the dairy system by 4.8 t CO₂ equivalent. This resulted in a new annual shadow cost to the dairy herd of approximately £20 830 per annum (or 1.94 p/kg milk) a reduction of £127 per annum. The SPC REV for milk yield was calculated to be £0.012 per kg of milk per cow per annum, or £3.64 per genetic standard deviation unit. Adding this to the current £PLI would result in a relative weight in the index of 6% compared to the other production and functional traits already incorporated in £PLI.

Conclusions This example shows a method by which a non market or shadow environmental value can be incorporated into selection indices using increased milk yield to reduce the herd size required to maintain herd output as the goal trait. A similar framework could be applied to other traits under selection to estimate a new suite of REVs that consider combinations of market and non market traits (positive and negative). Note also that many of the other traits included in £PLI, such a lifespan and health and fertility traits, will have an additional favourable impact on GHG emissions by improving overall system efficiency.

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Figure 1 CH₄ and N₂O emissions from the milking herd and young stock expressed in t CO₂ eq for a defined dairy

Figure 2 Shadow price of carbon (SPC) to the herd in £GBP per annum when milk yield is improved.

Effects of dietary protein concentration on energetic efficiency and methane emission in lactating dairy cows

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Introduction A series of studies have been undertaken to examine effects of dietary protein source and concentration on milk production, energy and nitrogen (N) utilisation efficiencies and fertility in dairy cows. The present study evaluated effects of dietary crude protein (CP) concentration on energetic efficiency and methane emission in lactating dairy cows.

Materials and methods Forty seven first and 40 multi-lactation Holstein dairy cows were used in a continuous design study (from week 1 to 44 of lactation) to examine effects of dietary CP concentration on animal performance and nutrient utilisation. The CP concentrations evaluated were 180, 150 and 120 g/kg DM in mixed diets containing 450 g/kg DM of forage (60% grass silage and 40% maize silage (DM basis)) and 550 g/kg DM of concentrates. Concentrate supplements (n = 3) consisted of different proportions of the same ingredients (barley, wheat, sugar beet pulp, citrus pulp, soya bean meal, rapeseed meal and molasses). Full details of experimental design, animal, diets and managements are reported by Law et al (2008). During early (70 to 90 days), mid (150 to 170 days) and late (230 to 250 days) lactation, the same 4 cows and 4 heifers from each treatment were transferred to metabolism units for 8 days with measurements of feed intake and faeces and urine outputs during the final 6 days. Immediately following completion of digestibility measurements, all cows were transferred to indirect respiration calorimeter chambers for 3 days with gaseous exchange measured during the final 2 days. Live weight was recorded at the beginning and end of these measurements and milk yield and fat, protein and lactose concentrations in milk were measured daily. The data were analysed as a one way ANOVA, with period as block.

Results There was no significant difference in live weight or fat, protein or lactose concentration in milk between the 3 diets, but increasing dietary CP concentration significantly increased DM intake (16.5, 18.4, 19.5 kg/d, s.e. 0.43, $P < 0.001$) and milk yield (20.6, 26.1 and 28.3 kg/d, s.e. 0.94, $P < 0.001$). Increasing dietary CP concentration significantly increased GE intake, energy outputs in urine and milk and heat production ($P < 0.001$, Table 1). Faecal energy output was lower with low than medium CP diets ($P < 0.001$), but there was no significant difference between medium and high CP diets. Dietary CP concentration had no significant effect on methane energy output, energy balance or energy partitioning between milk and body tissue (milk energy/ME intake and energy balance/ME intake). There was no significant difference in the efficiency of ME use for lactation (k_l) between the 3 diets, when using ME requirements for maintenance of Agnew *et al.* (2004). Increasing dietary CP concentration significantly reduced methane emission as a proportion of DM intake ($P < 0.05$) and milk yield ($P < 0.01$), but the differences between medium and high CP diets did not reach significance.

Table 1. Effects of dietary CP level on energy utilisation and methane emission					
	Dietary CP concentration (g/kg DM)				
	120	150	180	s.e.	Sig.
GE intake (MJ/d)	301.0	340.0	363.1	7.84	***
Faecal energy output (MJ/d)	84.8	94.0	93.5	2.59	*
Urine energy output (MJ/d)	11.8	13.3	14.4	0.28	***
Methane energy output (MJ/d)	20.1	20.3	21.7	0.63	
Heat production (MJ/d)	124.9	132.0	143.2	3.06	***
Milk energy output (MJ/d)	64.1	79.6	86.1	2.44	***
Energy balance (MJ/d)	-4.7	0.7	4.3	3.02	
Milk energy/ME intake (MJ/MJ)	0.354	0.376	0.367	0.0085	
Energy balance/ME intake (MJ/d)	-0.036	0.000	0.017	0.0153	
Efficiency of ME use for lactation (k_l)	0.551	0.579	0.563	0.0130	
Methane output/DM intake (l/kg)	31.1	28.2	28.2	0.83	*
Methane output/milk yield (l/kg)	25.7	20.9	20.4	1.13	**

Conclusions Reducing dietary CP concentration significantly decreased feed intake and milk production, but had no effects on energy partitioning between milk and body tissue or k_l . Increasing dietary CP concentration from 120 to 150 g/kg DM significantly reduced methane emission as a proportion of DM intake and milk yield, but further increasing dietary CP concentration to 180 g/kg DM had no effect.

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The effect of level of dietary fish oil inclusion on intake and methane emissions of beef steers

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Introduction Irish livestock account for 0.55 million tonnes of methane (CH₄) annually from enteric fermentation and manure management (EPA, 2006). However, recent studies have shown the potential to reduce ruminal methanogenesis through strategic dietary supplementation with polyunsaturated fatty acids (PUFA; Jordan *et al.* 2006). This is likely to be mediated, in part, through the unsaturated double bonds of PUFA acting as sinks for free hydrogen during ruminal biohydrogenation. Fish oil is acknowledged as the richest natural source of long chain n-3 PUFA such as eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids (Ruxton *et al.*, 2004). Despite this, there is little published information on the effect of dietary fish oil supplementation on *in vivo* ruminal methanogenesis in cattle. The aim of this study was to assess the effect of a novel concentrated n-3 PUFA fish oil, on the intake and CH₄ emissions of beef steers.

Materials and methods Six mature Limousin crossbred steers with a mean ($\pm$ S.E.) initial liveweight of 662 kg ($\pm$ 12.7 kg) were allocated at random to a replicated 3 x 3 Latin square design. Each period lasted 28 days. Animals were housed in slatted floor pens and individually fed using Calan Broadbent feed gates. Feed was offered in 2 equal portions at 08:00 and 16:00h daily. Steers were fed a 50:50 forage:concentrate diet, with barley straw as the forage source. Intakes were restricted to 95% of voluntary intake. Three concentrates were manufactured to supply fish oil at 0, 1 or 2% DMI and all diets were formulated to be iso-nitrogenous (14% CP DM). The fish oil contained 43% EPA and 19% DHA. Dry matter intake was measured daily. Forage DM, was determined daily by oven drying at 55°C for 72h and concentrate was sampled daily, bulked by treatment to provide three samples from each experimental period and stored at -20° C prior to subsequent analysis. Following a 21-day dietary adaptation period, daily CH₄ emissions were measured on all steers on days 23-27 of each period using the sulphur hexafluoride (SF₆) tracer technique of Johnson *et al.* (1994). The CH₄ and SF₆ samples collected from the steers were analysed by gas chromatography and adjusted for ambient concentrations. Statistical analysis was performed in accordance with the latin square design using the PROC MIXED procedure of SAS.

Results Dietary supplementation with 2% fish oil resulted in a decrease of both daily and intake corrected CH₄ production ($P < 0.05$). This was associated with a concomitant linear decrease in intake ($P = 0.0001$; Figure 1) in the animals fed at the 2% level. The greatest incremental decrease in both absolute and feed intake corrected CH₄ emissions was recorded between 1 and 2% dietary fish oil inclusion ($P < 0.05$; Figure 2).

Figure 1 Effect of diet on feed intake (kg DMI/day)

Figure 2 Effect of diet on CH₄ emissions

Conclusion Inclusion of fish oil at 2% DMI led to a reduction in daily and feed intake corrected CH₄ production. This reduction however, was associated with a 9.5% reduction in feed intake. Therefore, the effects of dietary inclusion of this high n-3 PUFA fish oil on animal performance and meat quality warrant further investigation.

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Effect of level of concentrate supplementation on methane emission of Holstein and Holstein-Jersey dairy cows

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Introduction Enteric fermentation in ruminants is an important source of methane (CH₄) production. In the UK, ruminants contribute approximately 20% of all CH₄ emission, with most arising from cattle. This presents a considerable challenge for the dairy industry in the battle against global warming, and reduced CH₄ emission is likely to be a major target for government policy within the next few years. The objectives of the present study were to evaluate the effects of level of concentrate supplementation and cross-breeding of Holstein cows with Jersey sires on CH₄ production.

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 for an 11 day 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 analysis of variance to examine effects of breed and dietary concentrate level, using experimental period as block.

Results and discussion There was no significant interaction between cow breed and concentrate level (Conc. L) on any parameter. On average across the 2 concentrate levels, cow breed had no significant effect on live weight or milk yield, but Jersey-Holstein cows had higher body condition scores ($P < 0.05$), DM intake ($P < 0.01$) and corrected milk yield (milk energy output/energy content per kg standard milk (3.1 MJ/kg), $P < 0.05$) (Table 1). Total CH₄ output and CH₄/milk yield were higher with Jersey-Holstein cows ($P < 0.01$), while cow breed had no significant effect on CH₄/DM intake, CH₄-E (CH₄ energy)/GE intake or CH₄/corrected milk yield. On average across the 2 breeds, cows offered the high concentrate diet had higher DM intake, milk yield and corrected milk yield ($P < 0.001$), but lower CH₄/DM intake, CH₄/milk yield, CH₄/corrected milk yield and CH₄-E/GE intake ($P < 0.001$). When relating CH₄/corrected milk yield to corrected milk yield and CH₄/DM intake to DM intake with a common constant, Holstein cows had a marginally larger coefficient in the former relationship (with corrected milk yield ranging from 13 to 30 kg/d) and a slightly smaller coefficient in the latter relationship (Table 2). These linear relationships indicate that cross breeding had little effect on proportional CH₄ production.

Table 1. Methane emission of Holstein and Jersey-Holstein dairy cows at 2 levels of concentrate supplementation

Concentrate level (kg/kg DM)	Holstein		Jersey-Holstein		s.e.	Significance	
	0.30	0.70	0.30	0.70		Breed	Conc. L
DM intake (kg/d)	14.6 ^a	16.6 ^b	15.2 ^a	17.8 ^c	0.34	**	***
Milk yield (kg/d)	18.2 ^{ab}	21.7 ^c	17.9 ^a	20.2 ^{bc}	0.71		***
Corrected milk yield (kg/d)	18.1 ^a	21.8 ^{bc}	19.9 ^{ab}	23.4 ^c	0.73	*	***
CH ₄ output (l/d)	462 ^a	466 ^{ab}	503 ^b	504 ^b	14.2	**	
CH ₄ /DM intake (l/kg)	31.8 ^b	28.4 ^a	33.2 ^b	28.3 ^a	0.90		***
CH ₄ /milk yield (l/kg)	25.9 ^{bc}	22.2 ^a	28.8 ^c	25.5 ^b	1.04	**	***
CH ₄ /Corrected MY (l/kg)	25.9 ^b	22.0 ^a	25.6 ^b	22.1 ^a	0.96		***
CH ₄ -E/GE intake (MJ/MJ)	0.068 ^b	0.061 ^a	0.071 ^b	0.061 ^a	0.0020		***

Table 2. Linear relationships between proportional CH₄ emission and corrected milk yield (MY) and DM intake

Holstein	CH ₄ /corrected MY = -0.980 [Corrected MY] + 45	R ² = 0.71	Eq. [1]
Jersey-Holstein	CH ₄ /corrected MY = -1.054 [Corrected MY] + 45	R ² = 0.54	Eq. [2]
Holstein	CH ₄ /DM intake = -1.088 [DM intake] + 47	R ² = 0.24	Eq. [3]
Jersey-Holstein	CH ₄ /DM intake = -0.982 [DM intake] + 47	R ² = 0.37	Eq. [4]

Conclusions Increasing dietary concentrate level reduced methane emission as a proportion of feed intake and milk yield. However, cow breed (Holstein vs. Jersey-Holstein) had no significant effect on methane output as a proportion of feed intake or energy corrected milk yield.

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Effect of spice supplementation on *in vitro* methane production using ground wheat as a substrate

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Introduction Ruminants lose about 8 to 12 % of their food energy as methane (CH₄). Eructation of CH₄ from ruminants may contribute to the climatic change and global warming. Reducing CH₄ production can be of direct economic benefit because it coincides with greater energy-use efficiency of feeds by the animal. To reduce CH₄, antibiotics have been used in addition to their use for growth and disease prevention. However, the European Union has banned the use of antibiotics in animal feeds from January 2006, so there is a need to find alternative methods to reduce CH₄ production. It has been shown that tannin and unsaturated fatty acid can play important roles to reduce CH₄ production in ruminants (Patra et al., 2006; Giger-Reverdin et al. 2003). Some spices are high in tannin and others are high in fatty acids. In the present study, the chemical composition of five spices and their role in reducing *in vitro* CH₄ production were assessed. Ground wheat (wheat) is a highly fermentable feed as it can produce large amount of gas, and it was therefore used as a substrate to compare the effects of spices on *in vitro* rumen fermentation in this study.

Materials and methods Samples of five spices (Cinnamon=Ci, Clove=Cl, Coriander=Co, Cumin=Cu and Turmeric=Tu) were analysed for ether extract (EE), total phenol (TP), condensed tannin (CT) and fatty acids by using a gas chromatograph (GC). The effects of adding spices at a fixed level of 30 mg/g wheat as the common substrate were compared with wheat alone (Control =Ct) on the *in vitro* CH₄ production at 24h in triplicate by using a completely randomised design. Rumen fluid (RF) was obtained from 2 fistulated sheep just before feeding of fixed amounts of hay plus concentrate at 2:1 ratios. RF was mixed with the pre-warmed buffer at 1:4 ratio under CO₂ to prepare inoculum. Samples of 0.4g wheat 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. Syringes were attached with these tubes containing RF plus wheat to collect gas. Stopcocks were used to control the gas entry. Total gas volume was estimated by the displacement of pistons during incubation. The gas collected in each syringe was then analysed for CH₄ by using a GC equipped with a Flame Ionisation detector. The data were analysed by using General Linear Model of Minitab to compare spices for their composition and effect on CH₄ production from wheat at P<0.05.

Results Significant differences were observed between spices for different chemical components (Table 1). Co and Cu were higher in EE (P<0.001), Cl for TP (P<0.001), and Ci and Tu for CT (P<0.01). Unsaturated fatty acids (UFA) were higher in Tu (P<0.001), followed by Co and Cu. Methane production was lowest in the presence of Co, followed by Tu and was similar between Cl and Cu (Figure 1). Methane production was highest in control followed by Ci. The higher EE and UFA in Co might have caused lower CH₄ production. The higher UFA and CT contents of Tu also might have helped Tu to reduce CH₄ production. Though UFA was lower in Cl, higher TP might have helped Cl to reduce CH₄ to a similar level of Cu. Due to lower EE and UFA, reduction of CH₄ production in the presence of Ci was lower compared to other spices.

Table 1 EE (mg/g), TP (mg/g GE), CT (mg/g CE) and fatty acid (mg/g EE) content of spices

Spices	EE	TP	CT	SFA	UFA
Ci	34	73	53	557	443
Cl	73	168	27	866	134
Co	155	11	6	137	863
Cu	146	19	10	320	680
Tu	26	22	39	112	888
SEM	1.81	19.5	6.1	94	103
P <	0.001	0.001	0.008	0.001	0.001

GE = Gallic acid Equivalent; CE = Catechin Equivalent

SFA = Short chain fatty acid

Figure 1 Effect of different spices on methane and total gas production

Conclusions Methane production was lower in the presence than the absence of spices (Control). Among the five spices Co was found to be better than other spices to reduce CH₄ production from ground wheat. While higher UFA in Co, Cu and Tu might have reduced CH₄ by capturing free hydrogen during potential bio-hydrogenation, the higher TP in Cl might have reduced CH₄ by restricting the process of methanogenesis. Careful selection and combination of these spices in our future studies may allow us to manipulate rumen degradation and CH₄ production in ruminants.

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Are Scottish Blackface ewes more resistant to nematode parasite infection than Greyface ewes?

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Introduction The extent of periparturient relaxation of immunity (PPRI) to gastrointestinal nematode parasites is sensitive to metabolisable protein (MP) scarcity but also varies between breeds of sheep (Houdijk, 2008). For example, under *ad libitum* feeding, Scottish Blackface ewes had a lower extent of PPRI than Greyface ewes (Zaralis et al., 2008). Such between-breed variation in PPRI may not necessarily be associated with genetic resistance *per se* but could arise from a higher nutrient demand of the more productive Greyface ewes (Houdijk, 2008). This experiment was designed to test the hypothesis that if the extent of PPRI has only a nutritional basis, then the sensitivity of PPRI to MP scarcity will not differ between the breeds when MP feeding is adjusted for between-breed differences in MP demand.

Materials and methods Twin-rearing Scottish Blackface ewes [(BF); n=36; mean body weight =57.6kg; s.e.=0.70kg] and Greyface ewes [(GF); n=36; mean bodyweight =69.1kg; s.e.=0.73kg] were trickle infected with 10,000 *Teladorsagia circumcincta* infective larvae 3 times a week from day₋₄₀ onwards (day₀ is parturition). From day₋₂₄ until day₃₁, ewes were restrictedly fed at 0.9 times metabolizable energy requirement, and fed at either 0.8 (LP) or 1.3 (HP) times their estimated MP requirements (AFRC, 1993). These requirements were 85 and 120g MP/day during late pregnancy and 230 and 295g MP/day during lactation for the BF and GF ewes respectively. Ewes and their lambs were weighed weekly and within 12h post lambing. Ewe faecal egg count (FEC, in eggs per gram (epg) fresh faeces) was assessed twice weekly as an indicator for PPRI and transformed via log(FEC+1) for statistical analyses. Litter average daily gain (ADG; in g/d) was estimated by linear regression. Ewe and litter body weight and FEC were analysed through repeated measures 2 x 2 factorial ANOVA. Post parturition ewe body weight, litter birth weight and ADG were analysed using a 2 x 2 factorial ANOVA.

Results Feeding treatment and breed did not interact for post parturition ewe body weight ($P=0.268$). However, HP ewes were heavier than LP ewes (58.7 vs 57.1kg; s.e.d. 0.68kg; $P=0.025$). During pregnancy and lactation, the effects of feeding treatment, breed x feeding treatment, and time x breed x feeding treatment, on ewe body weight were not significant ($P>0.410$). Figure 1 shows the backtransformed mean FEC and litter body weight. Feeding treatment and breed interacted for litter birth weight ($P=0.005$). HP litters were heavier at birth than LP litters in GF ewes only (See Figure 1b). The interaction effects of breed x feeding treatment, and time x breed x feeding treatment on litter body weight during lactation were not significant. However, time and breed ($P<0.001$), and time and feeding treatment ($P<0.001$) interacted on litter body weight. This was reflected in differences in average daily weight gain; GF litters grew faster than BF litters (615 vs 444g/day; s.e.d. 15.1g/day; $P<0.001$), and HP litters grew faster than LP litters (616 vs 453g/day; s.e.d. 15.1g/day; $P<0.001$). During late pregnancy, all first and second order interaction effects on FEC were not significant. BF had lower FEC than GF ($P=0.018$) and LP ewes had higher FEC than HP ewes ($P=0.08$). However, breed and feeding treatment interacted on FEC during lactation ($P=0.05$). MP scarcity increased FEC in GF ewes ($P<0.001$) but not in BF ewes ($P=0.581$).

Conclusion The absence of an interaction between feeding treatment and breed on litter weight during lactation suggests that similar extents of nutritional scarcity were imposed between LP and HP ewes in both breeds. Hence, the lower extent of PPRI of LP-BF ewes relative to LP-GF ewes supports the view that Scottish Blackface ewes could be genetically more resistant to nematode parasite infection than Greyface ewes.

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Figure 1 Backtransformed mean ewe faecal egg count (eggs per gram of faeces, epg, with 95% confidence interval) and mean litter body weight of Greyface and Scottish Blackface ewes fed at 0.8 (LP) or 1.3 (HP) of their estimated MP requirement.

Faecal dry matter and nematode infection in parasite-resistant Merino sheep in Australia

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Introduction Scouring (diarrhoea) is a major concern for sheep producers as the accumulation of faecal material (dags) around the breech pre-disposes sheep to flystrike. Scouring occurs when the consistency of faeces is fluid with a low percentage of dry matter. In temperate areas such as the southern half of Australia, New Zealand and the United Kingdom, scouring is associated with ingestion of parasitic nematode larvae, mainly *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* (Larsen et al., 1994). Breeding sheep to be resistant to these nematodes is a sustainable parasite-control strategy due to reduced reliance on chemical treatment. However, in adult sheep, scouring appears equally prevalent in resistant animals and, in some environments, is even more severe than in susceptible sheep (Karlsson et al., 2004). In this experiment, we investigated how faecal dry matter (FDM) in sheep from a flock bred for resistance to parasitic nematodes changed when challenged with infective larvae. We expected that FDM would be lower in challenged sheep compared to unchallenged controls, and FDM would also be lower in sheep with high dag scores compared to sheep with low dag scores.

Materials and methods The sheep were from the parasite-resistant Rylington Merino line bred by the Department of Agriculture and Food Western Australia. Sheep from this line are selected for parasite resistance on the basis of BLUP estimated breeding values (EBV) for low faecal worm egg count (FWEC). Forty rams aged 20 months were selected from this flock on the basis of EBV for FWEC and dag scores such that there were 10 rams in each of the following four groups – 1) low FWEC, low dag score, 2) low FWEC, high dag score, 3) high FWEC, low dag score and 4) high FWEC, high dag score. These rams were treated with anthelmintic, then housed indoors in individual pens and infected daily with 1000 third stage larvae for six weeks, consisting of equal numbers of *T. circumcincta* and *T. colubriformis*. A fifth group of ten rams was kept uninfected as a control. Each week, at least ten grams of faeces was collected from each sheep, weighed and then dried in an oven at 90°C to determine the percentage of faecal dry matter (FDM). After six weeks, all sheep were euthanased and the number of adult and immature worms was counted in an aliquot from the contents of both the abomasum and small intestine. Repeated measures ANOVA was used to assess differences in FDM between groups and two-way ANOVA was used to analyse total worm counts ($\log_{10}(x+1)$). Results FDM was lower ($P<0.05$) in sheep with high dag scores (HDS) than in control sheep from 7 days after larval dosing commenced until the end of the experiment. FDM in LDS sheep was lower ($P<0.05$) than in sheep with low dag scores (LDS) at 14, 21 and 35 days after dosing commenced (Figure 1). At 14, 28 and 35 days LDS sheep had lower ($P<0.05$) FDM than control sheep. Within each dag score category, there was no difference in FDM in sheep with high or low FWEC. Low FWEC sheep had lower total numbers of *T. circumcincta* than high FWEC sheep ($P<0.05$; Table 1). There were no differences in worm numbers between LDS and LDS sheep (Table 1). There were substantially higher numbers of *T. circumcincta* than *T. colubriformis* in all sheep

Figure 1 FDM (means $\pm$ s.e.) in rams with high dag scores (HDS; $n=20$), low dag scores (LDS; $n=20$) and controls ($n=10$)

Table 1 Total worm counts (geometric means with 95% confidence intervals) for sheep with low or high FWEC EBV (each $n=20$) and low or high dag scores (each $n=20$).

*Differences between low and high FWEC significant ($P<0.05$).

	Total worms	
	<i>T. colubriformis</i>	<i>T. circumcincta</i>
High FWEC	47 (15, 157)	1302 (891, 1903)*
Low FWEC	11 (4, 38)	378 (118, 1214)*
High dag score	27 (9, 85)	510 (184, 1421)
Low dag score	20 (6, 74)	963 (452, 2054)

Conclusions Dag scores observed in adult, immune sheep are related to a reduction in FDM caused by challenge with nematode larvae, and not necessarily associated with a large FWEC. As a result, sheep with a low FWEC are likely to scour just as badly as or even worse than sheep with a high FWEC. Within the Rylington Merino parasite-resistant flock, all sheep are highly resistant to *T. colubriformis* and the majority of the variation in FWEC seems to derive from resistance to *T. circumcincta*.

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Unravelling some mysteries of porcine respiratory and reproductive syndrome virus (PRRSV) infections: new insights from modelling studies

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Introduction Porcine reproductive and respiratory syndrome (PRRS) is an endemic pig disease in most European countries, causing respiratory distress, fever and growth reductions in growing pigs and increased litter mortality in sows. The disease is characterised by exceptionally long-term viral persistence within the host, a weak innate host immune response and delayed adaptive host immune response, and large between animal variation in the immune response (Murtaugh *et al.*, 2004). Although numerous *in-vitro* and *in-vivo* studies produced valid insight into the fine details of the virus dynamics and its interaction with the host's immune response, several fundamental questions concerning the role of diverse immune components and host genetics remain unanswered. In this study mathematical models were developed to investigate the role of diverse processes caused by the virus or the immune response on the infection characteristics.

Materials and methods The mathematical models are described by systems of ordinary differential equations representing a number of processes involved in the interaction between the virus and the immune response, as illustrated in Figure 1: the virus targets primarily differentiated macrophages (m^+) and, after their infection, causes apoptosis (self-destruction) of the infected as well as of neighbouring macrophages. The cellular and humoral immune response is represented by cytotoxic T lymphocytes (CTLs, z) and by virus neutralizing antibodies (w), respectively. Diverse models were analysed, starting with the virus dynamics without host immune response and no distinction between types of macrophages, and gradually increasing complexity by stepwise inclusion of additional processes.

Figure 1: Schematic presentation of the model

Results In the absence of an immune response, the infection is predicted to either clear rapidly or persist forever. Restricting the virus target cells to differentiated macrophages increases the chance for clearance of the infection and can cause reductions in virus load in the absence of an immune response. Apoptosis of non-infected in addition to infected macrophages does not affect the conditions for persistence of the infection, but affects the number of infected cells and virus load in the long-term. The host immune response makes conditions leading to persistent infections more stringent. The course of infection and long-term infection status depends on the relative balance between the cellular and humoral immune response. According to the model, a strong and sustained cellular immune response would be required to clear the infection. A strong humoral response, although not sufficient for clearing the infection on its own, enhances the clearance rate of the infection and affects the severity of infection both at the acute and persistent stage of the infection. The model further suggests that animals with strong and balanced proliferation rates of immune cells have generally lower viral loads and manage to clear the infection faster.

Conclusions The mathematical models, although simple, enhance the understanding of the role of diverse processes involved in the interaction between the virus and the host immune response on infection characteristics. The model predictions are in line with experimental observations and quantitatively support emerging hypotheses from *in-vitro* or *in vivo* experiments. An accurate quantitative understanding of the viral pathogenesis and immune control would require knowledge of proliferation and death rates of the represented cell types – and of their variation between individuals.

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A comparison of the gut microbiota in indoor and outdoor reared pigs

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Introduction The bacterial populations present in the porcine gut play an important role in pig health. A number of factors may influence these populations including diet, medication and environment. To identify what effects rearing environments have on the gut microbiota, this study compares the numbers of four common groups of gut organisms – enterococci, *Bacteroides* spp., lactobacilli and coliforms – in faecal samples taken from pigs reared either indoors or outdoors. The presence of three zoonotic bacteria – *Campylobacter jejuni*, *Clostridium difficile*, and Verocytotoxigenic *Escherichia coli* O157 – was also assessed to determine whether rearing environment might influence the threat of zoonotic infections.

Materials and methods Piglets (n=24) were farrowed either outdoors or indoors. At 4 weeks of age all piglets were weaned indoors where they remained until slaughter. Faecal samples were collected from 12 indoor pigs and 12 outdoor pigs at 4, 7, 14 and 23 weeks of age. Enterococci and lactobacilli plate counts were performed on kanamycin aesculin azide agar and LAMVAB agar respectively, and incubated aerobically for 48 h at 37°C. Plate counts of *Bacteroides* spp. were performed in an anaerobic cabinet on bile aesculin agar and incubated anaerobically for 48 h at 37°C. Coliform counts were carried out using the most probable number method which involved inoculation in MacConkey broth and aerobic incubation at 37°C for 24 h, followed by confirmatory subculture in brilliant green bile broth and aerobic incubation at 44°C for 48 h.

For molecular analysis of zoonotic bacteria, DNA was extracted from each sample using a phenol: chloroform based method. PCR amplification of specific genes was used to detect the three zoonotic bacteria in each sample. The genes targeted were: the *hipO* gene of *C. jejuni*, the toxin A gene of *C. difficile*, and the *eaeA* gene of VTEC O157. Statistical analysis of the results was carried out with a Mann Whitney *U* test (SPSS version 11.0).

Results Considerable variation was observed in the populations of the common gut bacteria between weeks. However, there were few statistically significant differences between indoor and outdoor samples. There were no significant differences in the occurrence of zoonotic pathogens between environments. Minimal numbers of *C. jejuni* and VTEC O157 were detected, however a number of samples were positive for *C. difficile* (table 1). These numbers decreased with age.

Table 2 Statistical significance of difference in counts between weeks. P<0.05 is significant. NS = not significant.
↑ = significant increase, ↓ = significant decrease

Weeks	Enterococci		Bacteroides		Lactobacilli		Coliforms	
	Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor
4 – 7	↓ P<0.001	↓ P<0.05	↑ P<0.05	↑ P<0.01	↓ P<0.01	↓ P<0.001	↓ P<0.001	↓ P<0.001
7 – 14	↑ P<0.001	↑ P<0.001	NS	NS	NS	↑ P<0.001	NS	NS
14 – 23	↓ P<0.001	↓ P<0.001	↑ P<0.05	NS	↑ P<0.001	NS	NS	↑ P<0.001

Conclusions Rearing environment may affect the gut microbiota in pigs, but other factors (in particular diet, and/or age) clearly also play an important role. Pigs reared exclusively indoors retained higher populations of lactobacilli than pigs reared outdoors. This may confer a health benefit on indoor reared pigs. Interestingly, asymptomatic *C. difficile* colonisation was common in young pigs but decreased with age. Similar findings have been reported in humans (Donta & Myers, 1982).

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Zinc oxide inhibits enterotoxigenic *Escherichia coli* K88 growth in culture and reduces the inflammatory response of porcine intestinal epithelial cells to infection

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Introduction Pharmacological levels of zinc oxide in the post-weaning piglet diet reduce the incidence and severity of diarrhoea, in particular that caused by enterotoxigenic *Escherichia coli* (ETEC) K88 (Owusu-Asiedu *et al.* 2003). A previous *in vivo* genomic study (Sargeant *et al.* 2008) identified several genes differentially expressed in the small intestine of ETEC-challenged piglets when fed a zinc oxide-supplemented diet. This included decreased expression of several genes involved in the inflammatory and innate immune response. It has been reported that ZnO reduces adhesion and internalisation of K88 to cultured human enterocytes, counteracting the up-regulation of inflammatory cytokines caused by ETEC infection. However, this effect was not due to growth inhibition of ETEC K88 in ZnO (Roselli *et al.* 2003). The objective of this study was to determine whether zinc oxide shows the same mode of action in protecting porcine intestinal cells against ETEC K88 as has been demonstrated in human cells, providing an explanation for *in vivo* findings.

Methods Intestinal porcine epithelial cells (IPEC) J2s were maintained in antibiotic-free medium, (Schierack *et al.* 2006). ETEC K88 was grown on Luria Broth (LB) agar plates and in both LB and IPEC medium. Concentrations of 0, 50 μ M, 100 μ M, 200 μ M, 500 μ M and 1mM zinc oxide dissolved in HCl were used for all cell assays. Cellular viability was determined after two hours of ZnO treatment using an MTT colorimetric assay. ETEC K88 growth was determined by optical densitometry every hour, and confirmed by viable plate counts. IPEC J2 cells were grown to confluency in 6-well plates, and treated with zinc oxide (0 to 1mM), either with or without ETEC infection at a multiplicity of infection of 100. After 2 hours, cells were washed and used for adhesion assays or RNA extraction using TRIzol. Adhesion assays were performed by removing all non-adhered bacteria, lysing IPEC J2s with 1% Triton X-100 and plating out serial dilutions of lysate. Extracted RNA was reverse transcribed and used for real-time PCR on genes of interest. ANOVA was used in SPSS v14 to determine significant differences between ZnO treatments.

Results Zinc oxide greater than 200 μ M inhibited ETEC K88 growth, with no growth after 6 hours at 1mM. However, zinc did not have a bactericidal action, as ETEC cells remained viable once plated onto LB agar. Above 100 μ M ZnO, IPEC J2 viability decreased as ZnO increased, with only 48% ($\pm$ 2.3% SE) of cells exposed to 1mM ZnO for 2 hours remaining viable. No significant difference in bacterial adhesion to IPEC J2s was seen between zinc treatments. ZnO treatment of IPEC J2s did not alter expression of any of the genes of interest except for metallothionein (MT1) (Figure 1). ETEC K88 infection resulted in an increase in expression of several genes of interest, including TNF α and the chemokines CXCL2, CXCL6 and IL8. This increased expression was negated by increasing ZnO concentration (Figure 2).

Figure 1 Fold change in gene expression between ZnO-treated IPEC J2s in comparison to the no treatment control.

Figure 2 Fold change in gene expression in ETEC-infected IPEC J2s in comparison to the non-infected controls.

Conclusions Cell viability and growth assays have established that high levels of dissolved ZnO reduce ETEC K88 growth, although do not affect bacterial adhesion 2 hours post-infection, in contrast to previous findings (Roselli *et al.* 2003). However, the reduction in innate immune response gene expression to ETEC K88 infection is seen at concentrations of zinc below those affecting bacterial growth (0.1mM), and concentrations above this negatively impact on intestinal cell viability and are unlikely to be present in the small intestine. It is therefore likely that the positive effects of ZnO are not due to reduced presence or adherence of ETEC K88, but instead a more direct effect on host immune responses.

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The effects of sire genotype and the porcine circovirus type 2 vaccine on the growth performance of pigs from weaning to slaughter

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Introduction Crossbreeding is common practice in commercial pig production as one way to increase lean growth and improve meat quality (Visser et al., 2000). Reducing disease is another way to help improve growth rate in pigs. Porcine circovirus type 2 (PCV2) has devastated the pig industry in recent years. PCV2 infection increases mortality and reduces growth rate as it acts as an immunosuppressant thereby making pigs more susceptible to co-infections (Kixmüller et al 2008). Keeping disease at low levels has been achieved by improving husbandry standards, biosecurity, minimising presence of other pathogens and use of antibiotics. However vaccines against PCV2 are now available. The aim of this study was to investigate the effects of sire genotype and PCV2 vaccine on growth performance and body composition of pigs.

Materials and methods A 2x2 factorial design compared sire genotypes (Hampshire and Large White) and vaccination status (vaccinated or not). A total of 264 pigs were weaned at 28±4 days. Within each litter half of the pigs were vaccinated against PCV2 at three weeks of age. Pigs were then allocated to pens based on their genotype and vaccination status and balanced for weight and sex. Pigs were individually weighed at weaning and then at five, six, seven, 10, 12, 14, 15, 16, 17 and 22 weeks of age and prior to slaughter. Weekly pen feed intakes were recorded and feed conversion ratios (FCR) calculated throughout the trial. All pigs were given daily health checks (recording any ill health). Pigs were scanned for body composition at slaughter using an AUTOFOM scanner. A General Linear Model (Minitab version 14.0) was used to analyse any differences in growth performance, health performance and feed intake between the pigs.

Results Throughout the trial Hampshires had a higher average daily intake (ADI) and average daily gain (ADG) when compared to Large Whites (Table 1). There was no difference in FCR between genotypes. Vaccination had no effect on growth performance. However ADG at week 16 was higher ($P=0.069$) for vaccinated pigs compared to their non vaccinated counterparts (Figure 1). Although there were no significant differences in mortality between treatments, mortality as a whole was down by 9.3% compared to on farm mortality over previous years. Due to a faster growth rate Hampshires were 8.4 days younger at slaughter ($P<0.001$), however Large Whites were leaner at slaughter ($P=0.010$). Vaccinated pigs were on average 2.2 days younger at slaughter ($P=0.084$). No differences in carcass composition between vaccinated and non-vaccinated pigs were found. Large Whites required more antibiotic treatment throughout the trial ($H=4$; $LW=19$, $P<0.05$). Non-vaccinated pigs required more antibiotic treatment although this difference was not significant ($Vac=8$; $Non=15$, $P=0.274$). No two-way interactions were seen during the trial.

Figure 1 ADG 5-22 weeks of age

Table 1 ADI and ADG from weaning to finish

	H	LW	
	ADI	ADI	P
Stage	kg/day	kg/day	
Weaner	0.397	0.330	<0.001
Grower	1.216	1.106	0.001
Finisher	1.607	1.447	<0.001
	ADG	ADG	
Stage	kg/day	kg/day	P
Weaner	0.314	0.259	0.030
Grower	0.789	0.734	0.002
Finisher	0.859	0.747	0.001

Conclusions The results clearly show differences in performance between genotypes. Hampshires performed better than Large Whites in terms of ADG and age at slaughter due to a higher ADI and required less antibiotic treatment. There was no difference in FCR between genotypes. Although growth performance was better for Hampshires, Large Whites were in fact leaner at slaughter. There was no difference in body composition between vaccinated and non-vaccinated pigs. Disappointingly vaccination against PCV2 had no significant effect on growth performance or health status. However pigs moved buildings at 15 weeks of age so the higher ADG seen for vaccinated pigs at 16 weeks may indicate that non-vaccinated pigs were less tolerant to the stresses involved. A lower mortality rate was seen throughout the trial compared to previous years suggesting that on farm disease may have been lowered as a result of half the pigs on farm being vaccinated against PCV2. This research includes the preliminary results of a larger trial currently being carried out.

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Quebracho (*Schinopsis quebracho-colorado*) tannin for the treatment of sheep gastrointestinal parasites. Effect on faecal egg counts and lamb growth

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Introduction Farmers have become heavily reliant on anthelmintics for the control of nematode parasites. Due to the widespread development of anthelmintic resistance, alternative approaches for control of nematode parasites are needed. Cruden and Hovell (2008) reported that commercial lambs treated with quebracho tannin had reduced faecal egg counts and improved lamb growth. This paper reports a second *in vivo* study with quebracho tannins at higher dose rates in lambs.

Materials and methods Sixty male and female Suffolk cross lambs, of 7 to 8 months were chosen randomly from a group that had shown symptoms of internal parasites 14 days prior to the start of the experiment. They were kept in an 11 acre field, and were free to graze on grass, or kale. No concentrates were fed. They had free access to clean water at all times. Exactly one week before the first dosing, the lambs were brought inside to the sheep handling system where all husbandry activities were carried out. Lambs were individually weighed, and randomised to one of 5 groups with 12 lambs in each. The following week (20/10/07), all lambs were weighed, the first administration of wormer was given, and faecal samples taken from 3 identified lambs from each group. Lambs were weighed and faecal samples taken (same 3 lambs) at 1 week intervals and until 01/12/07. The Quebracho Tannin was the same as used by Cruden & Hovell (2008), and was mixed with water at 40°C, and orally administered by conventional dosing gun. The 5 treatments were:- 1) Dosed once 60g tannin. 2) Dosed twice with 60g tannin, the second dose given a week after the first. 3) Dosed once with 90g tannin. 4) Dosed once with a conventional anthelmintic. 'Zermex' active ingredient Moxidectin which belongs to the Ivermectin group. 5) Untreated Control. 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 faecal egg counts (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 Lambs dosed with Zermex had a lower faecal egg counts (FEC) than the Control – although not always significant statistically (Table 1). There were no significant effects of quebracho tannins on FECs, although there was the suggestion of a decline in FECs of Trichostrongyloidea. Treatment with Zermex gave the best ($P<0.05$) weight gain of 12.5 kg, (Table 2). There was a clear growth response to the tannin ($P<0.05$) despite there being no clear effect on FECs.

Table 1 Back transformed means and ranges of faecal egg counts per g fresh faeces. (n=3)

Treatment		Control	Tannin 60g once	Tannin 60g twice	Tannin 90g Once	Anthelmintic (Zermex)	SEM
Trichostrongyloidea	Week 0	2094	2065	1687	1932	1667	453
	Week 2	2104	1892	940	1888	13.6	1272
	Week 4	1738	1742	551	1321	0	2110
	Week 6	1361	1479	585	935	5.6	2080
Nematodirus	Week 0	394	496	409	443	527	109
	Week 2	400	460	383	386	0	59
	Week 4	400	415	323	3126	0	179
	Week 6	333	311	262	247	0	46
Other Species	Week 0	395	450	311	272	247	67
	Week 2	395	431	288	272	0	53
	Week 4	398	406	259	155	0	78
	Week 6	262	272	165	31.1	0	180

Table 2 Initial, and final weights of lambs, and weight gains. (n = 12)

	Control	Quebracho tannin 60g	Quebracho tannin 60g Twice	90g	Zermex'	SEM
Initial Weight (kg) (I)	38.1	36.8	36.7	36.9	38.0	0.31
Final Weight (kg) (F)	43.9	45.6	47.1	47.3	49.5	0.93
Weight Gain (kg) (F - I)	5.8	8.9	10.4	9.4	12.5	1.09

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. More work is needed to develop optimal dosing and management regimens with tannins.

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***In vitro* anthelmintic activity of Chicory extracts from plants of different vegetative stages on *Teladorsagia circumcincta* L3**

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Introduction The consumption of plants rich in plant secondary metabolites has been associated with improved resilience of parasitised hosts, i.e. their ability to perform under parasitic infection, compared to animals fed conventional feeds (Athanasiadou *et al.*, 2007). Furthermore, such bio-active plants can improve host resistance, which refers to the host ability to regulate gastrointestinal nematode establishment, development, fecundity and survival (Athanasiadou *et al.* 2008). *Chichorium intybus* L. (chicory) is such a bio-active forage and its potential anthelmintic activity is currently investigated as an alternative means to control parasitism in sheep production systems. In the present study we employed an *in vitro* assay to study a possible mechanism of anti-parasitic action arising from chicory, and to test whether this anthelmintic activity is affected by the vegetative stage of the plant.

Materials and methods Plant samples from chicory and *Lolium perenne* L. (perennial ryegrass, used as control) were hand-plucked from three different plots of each species at two time points: pre-grazing (May) and post grazing (July) in Scotland. Samples were dried at 60 °C for 48h, then ground and a 10% aqueous-extract was prepared from each species (5 g sample/50 ml water). The plant extracts were tested for their ability to inhibit the migration of *Teladorsagia circumcincta* third-stage infective larvae (L3). The parasites were isolated from monospecifically infected donor sheep. Infective larvae L3 were incubated either for two hours or overnight in one of four plant extract concentrations for each species: neat plant extract (100), a 1:1 dilution (50), a 1:4 dilution (25) or plain water (0). After incubation, L3 were allowed to migrate through a mesh (70µ diameter) into a water solution. Three replicates for each of the four extracts were used. The number of L3 having actively migrated through the mesh was counted. Larval migration percentage was calculated as $(Nm/(Nm+Nr)) \times 100$, where Nm= numbers of L3 larvae migrated, and Nr=number of L3 larvae retained by mesh. The larval migration rates were analysed using a 2x4 factorial ANOVA for the two plant species and the four extract concentrations at each time point.

Results Following the two-hour incubation in the plant extracts, larval migration was more strongly inhibited when L3 were incubated in chicory extracts ($P < 0.001$) compared to those incubated in perennial ryegrass in May (Fig. 1) but not in July (Fig. 2). There was a significant interaction between the species and their concentration. The neat chicory extract in May resulted in a higher larval migration inhibition ($P < 0.001$) compared to the 50, 25 and 0 concentration, while there was no differences for perennial ryegrass ones. Regarding the overnight incubation results, the migration ability of the larvae was reduced in both chicory and grass neat extracts (82%) compared to the control (97%) ($P < 0.001$) in May, while there was no difference between both species and their control in the July extracts.

Figure 1 Larvae migration (%) of *T. circumcincta* in different concentrations of the tested species pre grazing

Figure 2 Larvae migration (%) of *T. circumcincta* in different concentrations of the tested species post grazing

Conclusion Our results suggested that chicory may be responsible for reducing the motility of nematodes larvae during May. We have no explanation to offer for the between time of year differences, other than that the chicory plants may differ in their nutritional composition as a result of the different vegetative stage. The reduced motility in *in vitro* trials may be related to similar mechanisms *in vivo*, which may result in the inability of parasites to complete their life cycle within the host. It should be noted that in this study we only tested a single possible mechanism of action of the potential activity of chicory aqueous extracts against nematodes. Other extracts and other mechanisms of action should be investigated to disentangle the complex and possibly multi-dimensional effects of chicory on the resistance of parasitized hosts.

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Health status, production and reproduction performance in cattle herds as influenced by management

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Introduction The overall trend of the reproduction performance is a reduction since at least 20 years. In Belgium, the pregnancy rate at artificial insemination decreases about 1% per year (Opsomer *et al*, 2006). To obtain good reproduction performance, many requisite factors are necessary. Health status, nutritional status or production level are some well-known examples. Many studies review these factors and their impact on the reproduction performance. The aim of this work was to describe the impact of health status, production and management on the reproduction performance in Walloon farms.

Material and methods An inquiry was carried on in 2005 in Wallonia at the "Association Wallonne de l'Elevage". There were about 70 veterinarians, grouped in 18 units involved in the artificial insemination network who monitored the reproduction. The insemination network, spread in the entire Wallonia covered about 9000 dairy and/or beef farms. Within this network, 224 dairy herds and 51 beef herds were also supported by the technical and economy team of the association. One veterinarian from each unit was questioned during a full working day in order to provide reproduction data which were included in a data base along with the economy parameters.

Results The major problems accountable for partial failure in the reproduction performance for dairy or beef breed were nutrition and heat detection in both dairy herds and beef herds (about 30% for each problem). Then, in dairy herds, cysts accounted for 12% of the problems but they can be related also to nutrition. In beef herds, the third problem was metritis (12.6%). The major requests for veterinarians involved in the monitoring of the reproduction are summarized in Figure 1. In the beef herds, the age at first calving (AFC), the calving interval (CI) and the fertility index were negatively correlated to the herd size, ($r = -0.183$, $r = -0.267$, $r = -0.324$ respectively, $P < 0.05$). For the dairy farms, the CI only was correlated to the size ($r = -0.152$, $P < 0.05$). Table 1 shows the reproduction performance according to the production performance in the dairy and beef herds. The AFC was smaller when the annual milk yield increased (31.2 months for production < 5000 kg milk vs 28.0 months for production > 8000 kg milk). For CI an average production of about 6000-7000 kg seemed to be the best for cow fecundity (397 days). In the beef farms, the heifer and the cow fecundities seemed to be improved with the increase of production expressed as total live weight gain/ha, as indicated by the AFC which was 35.4 vs 30.5 months and by the CI was 469 vs 420 days for a production < 350 kg and > 650 kg of live weight/ha respectively. The dairy farmers seemed to invest more in reproduction than the beef farmers (> 70 € vs 40 € maximum).

Figure 1 Distribution of major acts required by farmers in the inseminator network

Table 1 Reproduction performance according to the production performance in the cattle herds

Production	Number	AFC (month)	CI (days)
Kg Milk Dairy herds			
>8000	29 (9%)	28.0 ±2.2	404 ±81
7000-8000	91 (28%)	29.1 ±4.8	426 ±29
6000-7000	99 (31%)	29.9 ±4.6	397 ±113
5000-6000	46 (14%)	30.4 ±3.3	405 ±112
<5000	59 (18%)	31.2 ±6.0	401 ±119
Kg weight/ha Beef herds			
>650	2	30.5 ±2.1	420 ±5
550-650	8	31.9 ±3.5	440 ±38
450-550	12	33.1 ±2.6	425 ±36
350-450	22	34.0 ±4.8	445 ±66
<350	7	35.4 ±4.7	469 ±43

Conclusion According to this study it appeared that quite good reproduction performance could be achieved, even if the size of the herd and the production level were increased. The management of nutrition, the heat detection quality and the control of the reproduction cycle seemed to be some of the major key factors to improve reproduction performance. Furthermore, even if these critical points were not well under control, progresses are on their way.

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Incidence of African swine fever and its socio-economic implications on pig production in Lagos state, Nigeria

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Introduction The significant contribution of pig production in the supply of high quality protein in Nigeria is evident in the rapid increase in number of pig farms witnessed recently. However, African Swine Fever (ASF) has since ravaged pig farms and consequently resulted in loss of farmers' investments especially in some parts of the Southwest Agro-ecological zone of Nigeria (Majiyagbe *et al.*, 2004; Otesile *et al.*, 2005; Olugasa and Ijagbone, 2007). Putting the growth of the enterprise back on track requires proper assessment of the socio-economic environment surrounding the spread of the disease with the aim of determining the extent of infection, the consequences in terms of mortality of animals and loss of revenue alongside identifying farm specific factors that could have contributed to the spread. This study thus investigated the incidence of ASF and its socio-economic implications on pig enterprise in the zone.

Materials and methods Data for this study were generated through a farm survey of 260 pig farmers selected by multi-stage sampling techniques from three agro-ecological zones (Western, Far East and East) in Lagos State. Data were analysed using descriptive statistics while chi square statistics was used to examine the relationship between ASF incidence and farm/farmer specific attributes.

Results Majority (91.5%) of pig farms in Lagos State are located in the Western Zone, predominantly in Oke-Aro farm settlement, which is a peri-urban location. The average stock size of the pig farmers was 149 pigs with crosses and Large White being the prominent breed stocked by the farmers, and they sourced their stock within the farm settlement. Many of the farmers (71.5%) did not have adequate access to timely warning information on incidence of ASF disease. Hence, the incidence of ASF was recorded in 88.4% of the farms (Table 1) and this resulted in infection rate of about 90% of the stock size and consequently, mortality rate of 79%, with revenue loss of about ₦485, 000 per farm (Table 2). There was a significant relationship between the incidence of ASF disease on pig farms and sharing of labourers and implements by farms, visit of farms by other farmers and customers; and transport of farm products and inputs together in the same vehicle ($P \leq 0.05$).

Table 1 Incidence of ASF Disease on Pig Farms

Incidence of disease	Frequency of Respondents	Percent
Yes	213	88.4
No	28	11.6
Total	241	100.0

Table 2 Impact of Disease on Swine Production

Age category	Stock size	Loss Value (₦)
Boar	9 (2.77)	73,690.47 (7,340.70)
Sow	18 (4.77)	180,061.05 (11,195.45)
Grower	39 (9.04)	138,832.21 (25,018.08)
Weaner	32 (8.86)	53,199.67 (10,341.56)
Piglet	20 (6.21)	40,070.51 (7,423.41)
Total	118 (39.41)	485,853.91 (3,2480.97)
	Mean	% of Stock Size
Total Stock Size	149	-
Number of Animals infected	134	89.9
Number of deaths recorded	118	79.2

Figures in parenthesis are standard errors.

Conclusion The study has shown a wide prevalence of ASF disease among the majority of pig farms and this has been contributed to by unhygienic practices among farmers. The disease incidence has resulted in enormous loss of investment and consequently a major limitation on sustained growth and the potential of the enterprise in income generation.

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Meta-analysis of the halothane gene effect on parameters of pig meat quality

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Introduction Technological meat quality has a significant economic impact and many publications have shown that pig meat quality is strongly influenced by the effect of genetic type (especially Hal and RN genes) and by rearing and slaughter conditions (Sellier and Monin, 1994). The quality of meat is generally assessed by measuring meat pH at different times post mortem, colour or drip loss. Our study consists on a meta-analysis based on 11 publications in order to assess the possibility of predicting the effects of halothane gene, sex, breed and slaughter weight of animals on three selected parameters: pH at 45 minutes post mortem (pH45), ultimate pH (pHu) and reflectance (L*-value).

Materials and methods A database containing data for $n = 2521$ pigs from 11 studies was assembled from the literature. The database contained information on halothane genotype and its effect on pig meat quality parameters as ultimate pH (pHu), pH at 45 minutes *post-mortem* (pH45) and reflectance (L*). Other experimental information were necessarily gathered and included into the database as sex, breed and slaughter weight of animals and sample sizes. In our study we compare two meta-analytical methods. The first one is the method of "Effect-size" which estimates the absolute difference between means of two levels. The combination of treatment difference estimates from all studies in order to give an estimation of the common "effect-size" is made by using a fixed effect model if the Cochran test of heterogeneity (Q-test) is not significant. Q-test follows a chi-squared distribution with $r-1$ degrees of freedom (r is the number of studies). Otherwise, the estimation of the common effect should be made by a random effects model, in which we include «Study» as random factor. The second meta-analytical method consists on the application of estimation models on the overall data and considering «Study» as a random factor. Data were analysed using a REML variance component analysis by a PROC MIXED of SAS software. To take into account differences between intra-study variances, we weighted all data by the inverse of variance corresponding to each study. The judgement parameters of the selected models quality were: R^2 (determination coefficient) and ρ -coefficient which represent the proportion of the total study variance that was due to the between-study variance (Hox and de Leeuw, 2003). ρ should not exceed 25%, otherwise, we should use a meta-regression analysis by including co-variables in the model to explain the persistent unexplained variance between studies. Meta-regression analysis corresponds to a multiple regression with backward elimination, and the selected model was the one with the lowest Akaike's Information Criterion (AIC) and also the lowest Residual Mean Square Error (RMSE).

Results Study results about differences between halothane genotypes were different for the three analysed parameters (pHu, pH45 and L*). The comparison of the two meta-analytical methods was carried out for ultimate pH only. The "Effect-size" method has shown a significant effect for halothane gene (NN vs. nn) and slaughter weight on pHu. With the conventional method, only halothane genotype had significant effect on pHu. No significant differences for pHu were found between Nn and nn genotypes with both methods. According to our results, pH45 was significantly influenced by halothane gene, breed and sex of animals, while L*-value was influenced by the same factors except but sex. However, the between-study variance was very important in all the selected models.

Table 1 Comparison of variances and judgement parameters for pHu, pH45 and L*.

Genotypes compared	pHu		pH45		L*	
	NN-nn	NN-Nn	NN-nn	NN-Nn	NN-nn	NN-Nn
Genotype differences	0.062 ± 0.01	0.048 ± 0.01	0.61 ± 0.06	0.194 ± 0.06	-4.27 ± 0.69	-0.43 ± 0.1
P	0.0005		0.0047		<0.0001	
Between-study variance	0.02292		0.0212		5.33	
Intra-study variance	0.000972		0.0094		1.29	
R^2 (%)	94		91		96	
ρ (%)	95		69		69	

Conclusions These results showed that halothane genotype had significant effect on all the three analysed parameters of pig meat quality. However, these conclusions should be confirmed by increasing the number of studies included in the meta-analysis in order to reduce the between-study variance which was high in all the selected models. The comparison between the two meta-analytical methods showed that ρ -coefficient was lowest in the "Effect-size" method.

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Novel epistatic QTL for chemical body composition and accretion as well as feed efficiency in pigs

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Introduction Most quantitative trait loci (QTL) studies have focussed on the identification of individual QTL effects (additive, dominance and imprinting) in the absence of interactions (epistasis). There are numerous reports in the literature for QTL associated with growth and body composition of pigs, however much less effort has focussed around the identification of epistatic QTL for these traits. Growth and body composition of pigs are probably influenced by numerous QTL located throughout the genome as well as interactions between QTL. Therefore the objective of this study was to investigate the contribution of epistasis to the genomic regulation of growth and body composition of pigs.

Materials and methods A genomic scan for epistatic QTL was based on data from a three generation full-sib design (Pietrain X crossbred dam line). In total, 386 animals were genotyped for 88 molecular markers covering 10 autosomes. Phenotypic data for chemical body composition in live animals, daily gain and feed intake was available for 315 F₂ animals. The analysis was based on a two stage approach. In stage one, a 5 cM scan was carried out across all genomic positions to pre-select potential candidate genes with epistatic effects. In stage two, a complete epistatic model was applied using a 1 cM scan around the pre-selected positions. All analyses were performed with QxPak software (Perez-Enciso and Misztal, 2004).

Results In total, 32 significant epistatic QTL pairs were identified (p value < 0.001): 11 for growth traits (daily gain, protein and lipid accretion), 6 for daily feed intake and food conversion ratio traits and 15 for chemical body composition (protein and lipid contents). Epistatic interactions were identified between QTL on SSC1, SSC2, SSC4, SSC6, SSC7, SSC8, SSC10, SSC13 and SSC14. No epistatic QTL were identified on or with SSC9. All types of fitted epistatic effect were identified in this study: additive x additive (aa), additive x dominance (ad), dominance x additive (da) and dominance x dominance (dd).

Table 1 Evidence of epistatic QTL pairs with the highest effect from the genomic analysis

Trait	LR	Q0 chr (cM)	Q1 chr (cM)	% var	Q01_aa ± SE ¹	Q01_ad ± SE ¹	Q01_da ± SE ¹	Q01_dd ± SE ¹
<i>Chemical body composition measured at 90 kg body weight</i>								
LC _{EB}	22.82	4 (130.1)	7 (100)	7.1	-1.080 ± 0.226	-0.019 ± 0.432	0.022 ± 0.324	-0.701 ± 0.666
PC _{FFS}	23.45	4 (130.1)	7 (100)	7.3	-0.259 ± 0.054	-0.007 ± 0.102	0.008 ± 0.076	-0.163 ± 0.156
PC _{EB}	23.15	4 (130.1)	7 (100)	7.3	0.027 ± 0.006	0.0004 ± 0.011	-0.001 ± 0.008	0.018 ± 0.017
LC _{EB}	24.84	8 (105)	6 (32)	7.7	-1.515 ± 0.309	-0.557 ± 0.613	-0.919 ± 0.645	-1.579 ± 1.181
PC _{FFS}	24.51	8 (105)	6 (32)	7.5	-0.360 ± 0.074	-0.124 ± 0.147	-0.201 ± 0.155	-0.359 ± 0.283
PC _{EB}	24.57	8 (106)	6 (32)	7.7	0.038 ± 0.008	0.014 ± 0.015	0.021 ± 0.016	0.036 ± 0.029
<i>Growth period 60-90 kg</i>								
FCR	27.67	7 (49)	13 (2)	8.4	0.244 ± 0.057	0.066 ± 0.080	0.201 ± 0.087	0.014 ± 0.125
<i>Growth period 90-120 kg</i>								
DG	33.68	1 (119)	2 (46)	10.3	3.83 ± 17.40	-121.00 ± 26.79	48.49 ± 26.27	-179.27 ± 41.43
PAR	32.06	1 (119)	2 (46)	10.2	1.03 ± 2.89	-19.59 ± 4.42	8.89 ± 4.30	-28.61 ± 6.80
FCR	26.76	2 (38)	14 (99)	8.3	-0.275 ± 0.082	0.301 ± 0.155	-0.600 ± 0.144	0.235 ± 0.263
<i>Growth period 120-140 kg</i>								
LAR	25.23	7 (18)	10 (9)	8.3	-44.45 ± 15.95	-41.12 ± 23.79	23.98 ± 27.63	182.74 ± 42.21

Values in bold represent significant epistatic effects. LR, likelihood ratio; LC_{EB}, lipid content of the empty body; PC_{EB}, protein content of the empty body; PC_{FFS}, protein content of the fat-free substance; FCR, food conversion ratio; DG, daily gain; PAR, protein accretion rate; LAR, lipid accretion rate; SE, standard error.

Conclusions This study is, to the best of our knowledge, the first to report epistatic QTL for growth and body composition of pigs. This study has shown that the genomic regulation of growth and body composition is a complex process involving many QTL throughout the genome as well as interactions between QTL. By accounting for epistasis a large number of QTL were identified which could not be detected from previous individual QTL analysis (Mohrmann *et al.*, 2006; Duthie *et al.*, 2008). Most of these QTL mainly exhibit their effects through interactions with other QTL. It is important to account for epistasis within QTL studies to prevent QTL remaining undetected and to prevent inaccurate estimates of QTL effects. The aa component is heritable and of interest for breeding to optimise the use of QTL information with marker assisted selection.

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The effects of three muscling Quantitative Trait Loci on growth patterns of crossbred lambs

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Introduction Texel Muscling QTL (TM-QTL) and LoinMaxTM (LM-QTL) are located on chromosome 18 in Texel and Poll Dorset sheep, respectively, and have been shown to positively affect muscling of the loin in crossbred lambs carrying one copy of the QTL (Macfarlane *et al.*, 2008, Masri *et al.*, 2009). MyoMaxTM (MM-QTL), found on chromosome 2 of Texel sheep, increases muscling and decreases fatness in lambs of different genetic backgrounds (Campbell and McLaren, 2007). No differences in live weights, carcass weights or growth rates have been associated with these QTL to date. However, there is further need to investigate the effects of the three QTL on growth patterns in commercial crossbred populations, where fast-growing slaughter lambs of good carcass weights and quality are a prerequisite.

Materials and methods Data were collected across three years from female and castrated male lambs produced by Mule ewes (Blue-faced Leicester x Scottish Blackface) that did not carry the muscling QTL, mated to rams that were heterozygous carriers of either TM-QTL (2006, Texel rams), LM-QTL (2007, Poll Dorset rams) or MM-QTL (2008, Texel rams). Therefore, lambs either carried one copy or no copy of the relevant QTL. Lambs were grazed on pasture until slaughter. Live weights were recorded at birth, at approximately 5, 10, 15 (or 18 in 2006) and 20 weeks of age, and pre-slaughter. A total of 49 non-carriers and 62 carriers of the TM-QTL were recorded, 96 non-carriers and 74 carriers of the LM-QTL, and 80 non-carriers and 68 carriers of the MM-QTL. Lambs were weaned at around 20 weeks of age and slaughtered several days later (age range 136 - 158d, live weight 23.8 - 51.4kg). A modified Gompertz growth function (Renne *et al.*, 2003) was fitted to the live weight and age data from each lamb, to estimate parameters with direct biological interpretation: *A*= estimated final body weight (kg); *B*= maximum live weight gain (kg /d); *C*= age at *B* (d). Multiple linear regression was performed within-year to determine the best model to explain these three growth parameters (*A*, *B* and *C*). The terms tested in the models included fixed effects of sex, litter size at birth and weaning, dam age, QTL status, date of birth and all two-way interactions. Significant fixed effects for each parameter in each year were fitted alongside random effects of sire and dam in a REML analysis in Genstat (Payne *et al.*, 2005) to produce least-squares means for carriers and non-carriers of the relevant QTL.

Results The modified Gompertz model fitted the data well across all years (average co-efficient of determination > 0.957) and the fit was not significantly affected by QTL status. QTL status did not significantly affect any of the Gompertz parameter estimates for any of the three QTL (*P*>0.05; Table 1).

Table 1 Gompertz parameter least-squares means for QTL carrier status

QTL	parameter	non-carrier	carrier	s.e.d.	P value
TM-QTL	A	41.8	40.8	0.80	0.21
	B	0.57	0.57	0.01	0.97
	C	37.5	36.5	0.71	0.19
LM-QTL	A	45.5	46.4	1.38	0.83
	B	0.35	0.34	0.01	0.91
	C	49.6	48.5	1.46	0.47
MM-QTL	A	38.9	39.3	0.81	0.17
	B	0.52	0.51	0.01	0.85
	C	38.5	39.7	0.69	0.19

There were indications that the interaction between litter size at birth (twin or triplet, all reared as twins) and LM- or MM-QTL status could significantly affect estimates of *A* (*P* < 0.01 for litter size × LM-QTL) and *B* (*P* = 0.08 for litter size × LM-QTL, *P* = 0.02 for litter size × MM-QTL), which requires further investigation.

Conclusions There was some indication that the growth path of lambs born as triplets and reared as twins may be affected by carrying one copy of the LM-QTL (lower maximum daily weight gain, but reaching a higher final weight), or MM-QTL (lower maximum daily live weight gain), but overall the muscling QTL investigated did not significantly affect growth patterns of the cross-bred lambs studied. This suggests that muscling could be improved in commercial crossbred lambs by using terminal sires that carry these QTL, without compromising growth rates.

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Comprehensive evaluation of the direct effects of LM-QTL on carcass traits in crossbred lambs

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Introduction LoinMax (LM-QTL) increases *M. longissimus dorsi* (MLD) area and weight by 11% and 8% respectively (Nicoll *et al.*, 1998). It was found to segregate in Australian Poll Dorset sheep (Banks, 1997) and has been mapped to chromosome 18 (Nicoll *et al.*, 1998). The UK sheep industry may benefit from using LM-QTL to increase yield of the highly priced loin region. However, before being utilised, the effects of LM-QTL need to be verified in a genetic background typical to the stratified structure of the UK sheep industry.

Materials and methods 169 multiparous Mule (Bluefaced Leicester × Scottish Blackface) ewes were inseminated with semen from 2 Poll Dorset rams heterozygous for LM-QTL, to produce crossbred lambs that were either carriers of a single copy of LM-QTL or non-carriers. Unexpectedly, one of the rams was also heterozygous for a QTL that affects overall carcass muscling (MyoMAXTM). This was accounted for by fitting MyoMAXTM status as a fixed effect in the statistical analysis. Lambs were weighed and X-ray computed tomography (CT) scanned at an average age of 113 days to measure loin muscling traits (MLD width, depth and area and 2D loin muscularity), leg muscling traits (width, depth and 2D muscularity; Jones *et al.*, 2002), predicted total carcass fat, lean and bone weights and muscle density in the ischium, 5th lumbar vertebra and 8th thoracic vertebra CT scans. Lambs were weighed and ultrasound scanned at an average age of 140 days to measure muscle and fat depths over the 3rd lumbar vertebra (UMD and UFD, respectively) and were then slaughtered. Carcasses were weighed and classified for fat cover and conformation scores based on the MLC classification scheme and then scanned using a video image analysis (VIA) system. The VIA traits obtained were predicted weights of primal cuts and trimmed primal (TP) cuts (shoulder, breast, leg, chump, loin,) and predicted weights of carcass saleable meat yield and trimmed saleable meat yield. Data were analysed using the GLM procedure in SAS (SAS Institute Inc., Cary, NC, USA) to determine the effects of LM-QTL on the traits measured. Fixed effects fitted were LM-QTL and MyoMAXTM carrier status, rearing rank and sex. Sire was fitted as a random effect. Either live weight (for in vivo measurements) or carcass weight (for measurements taken on the carcass) was fitted as a covariate.

Results Traits significantly affected by LM-QTL carrier status are shown in Table 1. MLD width, depth and area measured using CT scanning were significantly higher in lambs carrying a single copy of LM-QTL compared to non-carriers. UMD was also significantly higher in LM-QTL carrier lambs. Leg muscle depth was significantly higher in LM-QTL carrier lambs. LM-QTL carrier lambs showed significantly higher leg 2D muscularity than non-carriers. LM-QTL did not affect total carcass lean or fat weights or MLC classifications. Using current algorithms, VIA did generally not detect any significant LM-QTL effect on weights of saleable meat yield or weights of primal cuts, but the trimmed shoulder cut was significantly lighter in LM-QTL carrier lambs.

Table1 Significant (P<0.05) effects of LM-QTL on traits measured using ultrasound scanning (US), CT scanning and VIA.

	US	CT scanning					VIA
	UMD (mm)	MLD width (mm)	MLD depth (mm)	MLD area (mm ²)	Leg muscle depth (mm)	2D Leg muscularity	TP-shoulder (g)
Non-carrier	23.9	69.8	26.2	1577	79.2	4.7	3861
Carrier	25.1	70.9	27.4	1677	81.4	4.9	3819
sed	0.094	0.104	0.073	5.219	0.223	0.017	4.033
P-value	0.02	0.03	0.001	<0.001	0.05	0.04	0.04
%difference	4.98	1.59	4.54	6.35	2.83	3.68	-1.09

Conclusions LM-QTL significantly affected loin muscling characteristics of crossbred lambs carrying a single copy of the QTL in comparison to non-carrier lambs. This supports the previous reports about the effects of LM-QTL on loin muscling. Moreover, additional traits for which the effect of LM-QTL has not previously been reported were shown to be affected by LM-QTL: leg muscle depth and leg muscularity were higher in LM-QTL carriers than non-carriers. VIA could not distinguish any differences between LM-QTL carrier and non-carriers, with the exception of a slight negative effect of LM-QTL on the predicted weight of the trimmed primal shoulder cut.

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Evidence for segregation of a third gene with a major effect on ovarian function in Cambridge sheep

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Introduction Normal development of ovarian follicles leading to ovulation depends on interactions between the oocyte and somatic cells in the follicle as well as the general physiological *milieu*. The oocyte-secreted molecules bone morphogenetic protein 15 (BMP15) and growth differentiation factor 9 (GDF9) are essential for normal ovarian follicle growth and function (Shimisaki et al. 2003). Mutations in both BMP15 (*FecX^G*) and GDF9 (*FecG^H*) have been shown to be segregating in the Cambridge breed and ewes that are heterozygous carriers of either of these mutations have greatly increased ovulation rate while homozygous carriers of either mutation are sterile with a typical ovarian hypoplasia (Hanrahan et al. 2004). The sterility is due to failure of follicular development to progress beyond the primary follicle stage. However, not all cases of ovarian hypoplasia in Cambridge ewes could be explained by these mutations and sequencing of the entire coding regions for these genes did not reveal any mutations that could account for their typical sterile phenotype (Hanrahan et al. 2004). It was suggested, based on pedigree analysis of the four ‘unexplained’ sterile ewes, that another gene was responsible. The present report concerns the available evidence concerning this hypothesis.

Material and methods The data being reported here were obtained from ongoing observations on the self-contained flock of Cambridge sheep maintained at Teagasc, Athenry. The origin of this flock has been described (Hanrahan et al. 2004). Following the original discovery of ‘unexplained’ sterile sheep, subsequent selection of flock replacements was designed to increase the likelihood that the putative gene responsible was maintained in the flock. All animals surviving to weaning in this flock are genotyped for the BMP15 and GDF9 mutations known to be present in this population. Ovarian examination by mid-ventral laparoscopy is routinely done on all ewe lambs kept for breeding plus any ewe lambs suspected of having the sterile phenotype based on external examination (teat development). Additional ewes with ovarian hypoplasia that could not be explained by the genotype for either BMP15 or GDF9 have been identified. These observations were used to identify the male and female carriers of the putative gene. The female progeny from matings involving these carriers were used for proband analysis to test the hypotheses that the putative gene was X-linked. This involved calculating the probability for the observed incidence of ‘unexplained’ sterile daughters, after excluding the ascertainment cases, under the hypothesis that the putative gene was X linked and the alternative.

Results A total of 21 ewes with ovarian hypoplasia that cannot be explained by the genotype for BMP15 or GDF9 have been identified in this population, up to and including animals born in 2007. These cases were the offspring of 17 different ewes and 12 rams. The ‘unexplained’ sterile ewes and the carrier parents are classified according to genotype for BMP15

Table 1 BMP15 and GDF9 genotypes for ‘unexplained’ sterile ewes and for carriers of the putative gene

Genotype for		Ewe category		Ram [‡]
BMP15	GDF9	Sterile	Carrier	Carrier
<i>FecX⁺/FecX⁺</i>	<i>FecG⁺/FecG⁺</i>	7	4	3
<i>FecX⁺/FecX⁺</i>	<i>FecG^H/FecG⁺</i>	3	3	4
<i>FecX^G/FecX⁺</i>	<i>FecG⁺/FecG⁺</i>	4	7	3
<i>FecX^G/FecX⁺</i>	<i>FecG^H/FecG⁺</i>	7	3	1
<i>FecX^G/FecX⁺</i>	<i>FecG^H/FecG^H</i>	-	-	1

[‡] Note that in the case of rams only the first allele shown under BMP15 is carried since this locus is X linked;

progeny. The null hypotheses that the gene responsible is X linked was rejected ($P = 0.019$). The probability of observing 3 or fewer cases under the hypotheses of autosomal inheritance was 0.39. The rejection of the X-linked hypotheses is consistent that the fact that the putative mutation was not associated with BMP15 which is on the X chromosome. Based on the incidence of ‘unexplained’ sterile ewes over the period 1997 to 2007 the frequency of the putative gene in this population is approximately 0.3.

Conclusions Mutation in a gene other than BMP15 or GDF9 is responsible for the ‘unexplained’ cases of ovarian hypoplasia observed in this Cambridge flock. Thus, 3 loci with major effects on ovarian function are segregating in the Cambridge breed and the putative third gene is inherited in an autosomal fashion.

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Linkage disequilibrium in two divergent genetic groups of dairy cows using a high-density map of Single Nucleotide Polymorphisms

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Introduction A 50K bovine DNA array allows large scale genotyping of dairy cattle for around 50 thousand markers (Single Nucleotide Polymorphisms - SNP). This development opens the door to genomic prediction and selection, and also facilitates gene detection, mapping and association studies. Any such application requires knowledge of the Linkage Disequilibrium (LD) state of the markers. The objective of this work was to examine the LD in two divergent genetic lines of dairy cows genotyped with the 50K bovine DNA array.

Materials and Methods DNA extracted from 299 Holstein cows was used to determine genotypes in 54,001 SNP loci with the Illumina BovineSNP50™ array. These animals were from the Langhill lines research herd of the Scottish Agricultural College in Crichton, had calved between 1993 and 2005, and participated in a selection experiment conducted during that period, where 133 cows had been sired by average genetic merit bulls (control group) and 166 were daughters of high genetic merit sires (selected group). Data edits removed SNP loci with a major allele frequency greater than 0.95, call rate less than 65% and missing valid chromosomal assignment or position. The Hardy-Weinberg equilibrium (HWE) state was assessed in each SNP locus with a chi-square test. LD values were calculated both within and across genetic group for all possible syntenic SNP pairs as the squared correlation between alleles at two loci (Hill and Robertson, 1968).

Results After edits, 41,859 SNP loci (78% of the original total) were kept for analysis. The majority (96%) of these loci were in HWE ($P > 0.05$). The average LD, calculated across all SNP pairs and chromosomes, was 0.0427 (s.d. 0.0336), 0.0452 (s.d. 0.0445) and 0.0462 (s.d. 0.0458) for all, control and selected group cows, respectively. Average LD values were fairly uniform within different chromosomes, ranging from 0.0411 to 0.0517. Average LD values considering only SNP within 1 cM intervals were 0.0691 (s.d. 0.1082), 0.0705 (s.d. 0.1106) and 0.0746 (s.d. 0.1178) in the three datasets, respectively, and were slightly higher than estimates from another high-density map study using a 10K SNP array (Sargolzaei *et al.*, 2008). Figure 1 depicts these results by chromosome number.

Figure 1 Average LD for SNP loci in 1cM intervals, by chromosome; ALL=all, CON=control, SEL=selected group cows

Looking at adjacent SNP, overall average distance was 0.063 cM and average LD 0.125 in the three datasets. Of all possible SNP pairs, 53,487 (0.16%), 73,708 (0.22%) and 95,279 (0.29%) in the entire dataset and the control and selected group subsets, respectively, were in LD greater than 0.30, which is considered the minimum useful for mapping purposes and genomic selection (Sargolzaei *et al.*, 2008). A few SNP loci pairs (844, 984 and 1070 in the three datasets) were found in nearly perfect (>0.99) LD, suggesting that genotyping only one of them would be sufficient. The overall product-moment correlation of LD values calculated within the control and selected group of cows was 0.47 ($P < 0.001$). These correlations by chromosome number ranged from 0.30 (chromosome X) to 0.56 (chromosome 14).

Conclusion Different LD values were observed in the two selection lines. The group of cows that had been sired by high merit bulls were in slightly higher average LD. Analysing each genetic group separately revealed stronger LD than looking at the entire dataset. Results suggest that genetic selection has influenced the LD level in the studied population. Results can be used to support gene detection and genomic prediction studies.

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The effect of linkage disequilibrium on the estimates of Single Nucleotide Polymorphic effects

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Introduction The identification and exploitation of single nucleotide polymorphisms (SNP) associated with production traits present new opportunities for livestock genetic improvement. Often the identified SNP is not the causative mutation but rather is in some degree of linkage disequilibrium (LD). LD markers within 5cM can be considered as direct markers for the causative mutation because they are located close to the causative mutation (Dekkers, 2004). In a dairy herd, Farnir *et al.*, (2000) estimated that the average LD, measured as D' was 0.5 for loci pairs positioned within 5cM. Goddard *et al.*, (2006) estimated that LD measured as r^2 decreased rapidly as the physical distance between loci increased; at a separating distance of 0.5Mb the LD (r^2) was only approximately 0.2. The aim of this work was to use stochastic simulation to investigate the effect that the distance between the SNP and causative mutation had on the accuracy of estimating additive and dominance effects of the causative mutation.

Materials and methods FORTRAN90 was used to simulate a population with 3000 animals/generation, replicated 100 times. A single trait influenced by a single causative bi-allelic SNP was simulated with a total additive variance of 0.20 and phenotypic variance of 1.00. The SNP had an allele frequency of 0.5 and explained 10% of the total additive variance; polygenic variance=0.18, SNP variance=0.02 (additive effect = 0.2 and dominance effect = 0). Nine LD SNPs were simulated at distances of 0.01 to 5cM away from the causative SNP. Assuming 1 cM=1Mb, the r^2 value was interpolated from the figure presented by Goddard *et al.*, (2006) and the corresponding haplotype probabilities used to assign alleles in the base generation. The linked SNPs were also simulated to have allele frequencies of 0.5. Using ASReml (Gilmour *et al.*, 2002), datasets for each replicate and linked SNP were analysed using an animal model with the linked SNP fitted in the model as a fixed class effect. The SNPs were analyzed one at a time. The estimated SNP additive and dominance effects were calculated using the REML solutions, where the additive effect was half the absolute difference between the homozygote REML solutions and the dominance effect was the deviation from the mid-point between the homozygote classes. Results for each linked SNP were compared with estimates from the causative mutation using a Z test.

Results When the direct SNP for the causative mutation was fitted in the model, the estimated additive and dominance effects were not significantly different from the simulated effects of 0.2 and 0, respectively. As the distance between the linked SNP and causative mutation increased and LD decreased, estimates of the size of the additive effect also decreased. Figure 1 shows that to accurately estimate the additive effect of the causative mutation, the linked SNP needed to be within 0.02cM of the causative mutation, or alternatively have an r^2 of 0.88 or higher with the causative mutation. These results also showed that for all linked SNPs, the estimated dominance effect was 0, as simulated.

Figure 1 Estimates of the additive and dominance effects for linkage disequilibrium SNPs fitted as fixed class effects (error bars are 95% confidence intervals and x axis is not to scale)

Conclusions Linked SNPs positioned within 0.02cM of the causative mutation were able to accurately estimate the additive effects of the causative mutation with 3000 animals, no dominance and a favourable allele frequency of 0.5. This distance is smaller than the 5cM used to define linked SNPs. With different parameters, the required distance of the linked SNP to accurately estimate the additive effects of the causative mutation may vary. The potential benefits of linked SNPs decrease as the distance between the linked SNP and causative mutation increases and the ability to estimate the additive effect of the causative mutation decreases.

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Use of forage brassicas and bio-active chicory /plantain/clover mixtures for lamb finishing

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Introduction Traditionally UK lamb finishers have used forage brassicas to extend the season of supply of lamb beyond the grazing season. Retailers however can now import low-cost chilled lamb from New Zealand from December to June so financially U.K. supply extension is now more risky. Here, two trials are described to i) evaluate the ability of currently used forage brassicas to finish lambs in Nov/Dec, and to ii) compare the most promising ones with mixtures containing chicory, plantain and clover for finishing lambs in August /Sept. The latter having shown to be bio-active with anthelmintic effects.

Materials and methods Five 0.33ha plots of forage brassicas (kale (K), rape (R), stubble turnips (T), hybrid kale/rape (HB) and a rape/turnip (RP) mixture) were established on an upland (225 m.a.s.l.) old grassland site in Peeblesshire (Lat 56 degree N). Crops were sown on 9th July (kale on 8th June) and lamb performance evaluated from 3rd Nov to 12th Dec 2007 using 75 store lambs (32-36 kg). Lambs were weighed empty on arrival (day₀), given an anthelmintic, allocated to the plots balanced for initial live weight and breed (n=15), weighed again on day₃₀ and slaughtered on day₃₉. Lambs had access to mineral/vitamin powder, water and an area of grass runback of approximately 0.07ha. The abattoir recorded carcass weight, fatness and conformation, which were used with body weight data and an estimated carcass weight on day₀ to estimate daily bodyweight gain, killing out percentage and carcass gain. These animal data were used to assess effects of forage brassicas through ANOVA. In 2008 crops were sown earlier (30th May), and were a repeat of hybrid kale/rape, *Brassica oleracea/Brassica napus* (HB); hybrid kale/rape and chicory/plantain, *B. oleracea/B. napus* and *Cichorium intybus/Plantago major* (HB/CP); chicory/plantain/white clover, *C. intybus/P. major* and *Trifolium repens* (CP/WC); chicory/plantain/white clover and timothy grass, *C. intybus/P. major/T. repens* and *Phleum pratense* (CP/WC/T); and, chicory/plantain with white and red clover, *C. intybus/P. major* and *T. repens/T. pratense* (CP/WC/R). These were grazed between 18th Aug and 17th Sept with parasitised lambs. Lamb liveweight gain and dag score (5 point subjective score for faecal soiling of the breach area) were recorded for all lambs and strongyle faecal egg count (FEC) were recorded for 3 lambs per plot. Data was assessed through ANOVA; FEC were log-transformed and initial FEC was used as a covariate for final FEC.

Results In 2007 despite there being significant differences in daily gain on the crop, estimates of daily carcass gain were not significantly different between treatments as there were differences in killing out percentage (refer to Table 1). All crops grew well, the stubble turnips, being sown earlier than normal, had exceptional yields but lambs had difficulty utilising the crop when roots broke free of soil. The HB (cv. Swift) was chosen to compare with chicory/plantain mixes in 2008. The two plots containing HB outyielded the three chicory /plantain mixes (refer to Table 2). They showed significantly higher lamb liveweight gain, whilst it was observed that lambs on HB/CP preferred to eat HB to CP. After 44 days on the crop 90 % of HB and HB/CP lambs were in finished condition versus only 80% of the lambs on the other chicory/plantain mixes. Lamb daily gains on chicory/plantain mixes were lower than expected from previous work where gains over 250g/day were achieved. 2008 was a wet summer and estimates of dry matter content of the brassicas was 87g/kg vs. 67g/kg for chicory/plantain mix suggesting water content of the forages may have constrained intake and performance. Whilst the overall effect of forage approached statistical significance, lambs on the chicory /plantain mixes showed reduced FEC compared to lambs on brassicas (P<0.05 by contrast statement) and the mineral content of the forages was around twice as high. Crop treatment had no significant effect on dag score, but dag score reduced over time across feeding treatments. Dirty fleeces and dags, a common problem with late season lambs, were not seen.

Table 1 2007 results

Crops 2007	Fresh yield (t/ha)	Daily gain (g/d)	Carcass gain (g/d)	Killing out (%)
R	40	185	128	46.7
T	114	209	133	45.4
HB	51	246	143	45.1
K	58	210	132	45.7
RP	44	251	135	44.2
s.e.d		24	9.8	0.84
P-value		<0.05	NS	0.05

Table 2 2008 results

Crops 2008	Fresh yield (t/ha)	Initial FEC (epg)	Final FEC (epg)	Daily gain (g/d)	Final dag score
HB	55	227	959	184	1.4
HB/CP	41	253	656	215	1.4
CP/WC	39	223	335	130	1.4
CP/WC/T	34	139	425	102	1.8
CP/WC/RC	40	111	258	127	2.0
s.e.d.				24	
P-value			0.12	<0.05	NS

Conclusions Forage crops grazed in situ can add value and weight to lambs at low cost and improve efficiency of production by releasing grazing for ewes when lambs are marketed earlier. These UK trials with chicory /plantain mixes in a wet year have failed to show the exceptional gains typical of New Zealand trials but show promise as bio-active forages that reduce the need for worming and that improved trace element /mineral status of lambs. Further evaluation is justifiable.

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Investigation of the variation in lamb meat quality on three winter finishing systems

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Introduction Consumer purchasing decisions are, to a large part, governed by the level and consistency of meat quality and there is a need to identify systems of production that deliver consistent lamb eating quality through the winter months that will restore retailer confidence in British lamb outside the main grazing season.. The Vipond et al. (2005) report on lamb eating quality highlighted the deterioration in lamb flavour post-Christmas and recommended that dietary effects be investigated with a specifically designed experiment.

Materials and methods 180 Texel cross Mule lambs from one Shropshire farm were allocated to a three feed treatment x two finishing period study to investigate variation in meat quality by diet and date of slaughter. Lambs were finished in groups of 30 after a 6 to 7-week finishing period in either November (N) 2007 or March (M) 2008. The feed treatments were grass or grass silage (with compound supplementation where necessary) (G), stubble turnips (T) or *ad-libitum* compound feed (C). Lambs were weighed fortnightly until sale aiming to finish at 18 to 21 kg carcass weight and fat class 2/3L. Loins of 16 wether lambs from each treatment were compared with lambs from two control groups: grass-only fed British lamb (from the same Shropshire farm) (UK) slaughtered in November and New Zealand (NZ) lamb delivered, vacuum-packed and chilled, in May. Fresh samples were assessed for pH and colour (using standard CEILAB methodology) with the remainder being vacuum packed and frozen until required. Sensory analysis was carried out by trained assessors who ranked samples on 8-point category scales for tenderness, juiciness, lamb and abnormal flavour intensity, flavour liking and overall liking. Animal performance data were analysed using ANOVA with diet and finishing date as factors; carcass fatness and conformation data were analysed using the chi-squared test. Meat quality and taste panel results were analysed using ANOVA using type as a factor.

Results C lambs grew significantly faster, resulting in both heavier lambs at sale (44.8 kg cv. 41.7 and 41.1 kg for C, T and G lambs respectively, $p<0.001$) and a reduced average days to sale (50.5, 56.2 and 57.0, $p=0.004$) for C, T and G lambs respectively). Dressing proportion was highest for T and lowest for G lambs (0.457 cv 0.416, $p<0.001$). Slaughter date did not affect carcass conformation or fatness significantly but G lambs were leaner with poorer conformation. Overall growth rates, dressing proportion and days to sale were unaffected by slaughter date. Mean loin muscle pH varied between 5.5 (G in March) to 6.1 (NZ), an atypically high value usually caused by glycogen depletion pre-slaughter (Table 1). The meat from the two control groups was significantly darker than the other groups and the NZ lamb had a significantly lower hue value (redder) than all other groups and a significantly lower chroma (duller) than UK. NZ lamb was the most tender. For G and C lambs, there was deterioration in some quality attributes between November and March, most notably in abnormal flavour (which increased) and flavour liking and overall liking (which decreased). Compared with the UK controls, both groups of G and C lambs had weaker lamb flavour and, apart from the November-slaughtered G lambs, a significantly more pronounced abnormal flavour. The UK controls were preferred overall to any of these groups. On the other hand, both T groups had quality ratings on a par with the UK controls and, moreover, there were no significant effects of season of slaughter on this diet. In terms of overall liking, the most preferred were the two T groups and the UK controls; the least preferred groups were G and C slaughtered in March. The flavour changes were more important in determining overall liking than the changes in tenderness and juiciness between the two slaughter times, some of which favoured the March-slaughtered groups. But it is noteworthy that all lamb groups produced meat that was tender.

Table 1 Ultimate pH and some of the sensory ratings for the different treatments.

Variable	GN	GM	CN	CM	TN	TM	NZ control	UK control	LSD	P
Ultimate pH	5.77 ^b	5.51 ^c	5.75 ^b	5.59 ^{de}	5.71 ^{bc}	5.62 ^{cd}	6.13 ^a	5.63 ^{cd}	0.07	<0.001
Tenderness	5.22 ^b	4.94 ^c	4.48 ^d	5.26 ^b	5.40 ^b	5.17 ^{bc}	6.18 ^a	5.33 ^b	0.25	<0.0001
Lamb flavour	4.10 ^c	3.92 ^c	4.05 ^c	4.08 ^c	4.21 ^{bc}	4.49 ^{ab}	4.54 ^a	4.48 ^{ab}	0.31	<0.0001
Abnormal flavour	2.66 ^c	3.47 ^a	2.99 ^b	3.47 ^a	2.47 ^c	2.51 ^c	3.15 ^{ab}	2.37 ^c	0.33	<0.0001
Flavour liking	4.59 ^b	3.94 ^c	4.36 ^b	3.82 ^c	4.98 ^a	5.00 ^a	4.30 ^b	5.25 ^a	0.30	<0.0001
Overall liking	4.54 ^b	3.90 ^d	4.13 ^{cd}	3.85 ^d	4.90 ^a	4.85 ^a	4.27 ^{bc}	5.06 ^a	0.29	<0.0001

^{a,b,c,d} means within a row having different letters differ significantly ($p<0.05$) Sensory scores are on 8-point category scales (1=extremely tough/extremely disliked to 8=extremely tender/extremely liked)

Conclusions Stubble turnips provided a consistent diet that delivered a high level and consistency in lamb eating quality through the winter months. Later slaughter tended to reduce the assessors overall liking for grass silage and concentrate fed lambs. UK lamb compared favourably with this sample of NZ lamb but the latter had atypically high pH.

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The effect of genotype and diet on lamb meat quality from hill sheep systems

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Introduction Crossbreeding can be used in the hill sector to improve the efficiency of production. Carson *et al.* (2001) showed that ram breed substitution increased lamb output per ewe by 24% with concomitant improvements in carcass quality. However, information is required for a range of crossing sire breeds for the hill sector to enable the most effective crossbreeding programmes to be developed. Therefore, the primary aim of this study was to evaluate the effects of a range of lamb genotypes on lamb meat quality. Furthermore, a range of production systems (long-keep grass-based systems to short-keep concentrate-based systems) are used to finish hill lambs. Diet type (i.e. roughage- versus concentrate-based) can also affect lamb growth rates and carcass characteristics (Carson *et al.*, 2001). Consequently, a secondary aim of this study was to evaluate the effects of diet and its interaction with lamb genotype on lamb meat quality.

Materials and methods Over 2 years a total of 202 male lambs were sourced from six hill farms participating in a study investigating the relative merits of crossbreeding in the hill sector (Carson and Dawson, 2005). The lamb genotypes compared were as follows: Purebred Blackface (B×B); Swaledale X Blackface (SW×B); Cheviot X Blackface (CH×B); Lleyen X Blackface (LL×B); and Texel X Blackface (T×B). During the finishing period, lambs were allocated to the following diets: a low plane of nutrition (LOW, fixed allowance of dried grass pellets) or one of two high planes of nutrition (HIGH), consisting of a mixture of dried grass pellets and cereal-based concentrate (1:4) offered *ad libitum*. The concentrate component was formulated without or with oilseed rape (150 g/kg) (HIGH1 and HIGH2, respectively). Lambs were slaughtered at 42 and 50 kg, to enable treatment differences to be evaluated at different end-points. Instrumental meat quality measurements were determined on loin chops taken at 24 h post mortem and measurements of ultimate pH_U, cooking loss, Warner Bratzler Shear force (WBSF) and CIELAB colour parameters were determined using the methods detailed by Moss *et al.* (1993). Due to the unbalanced nature of the experimental design, the data were analysed using the GenStat REML (Residual Maximum Likelihood) procedure. The model tested main effects of lamb genotype, treatment diets and interaction, with fitted fixed effects for year, farm, lamb type (entire or castrate) and fat class.

Results The effects of lamb genotype and plane of nutrition on meat quality are presented in Table 1; there were no statistically significant interactions therefore only the main effects are shown. Lamb genotype had no effect on meat quality or colour parameters measured ($P > 0.05$). There were no effects of diet on instrumental meat quality ($P > 0.05$). Offering the concentrate diet containing oilseed rape (HIGH2) decreased lightness values (L^*) compared to the other dietary treatments ($P < 0.05$), and tended to decrease yellowness values (b^*) compared with the cereal-based concentrate (HIGH1) ($P < 0.1$).

Table 1 The effects of lamb genotype and treatment diet on instrumental meat quality and CIELAB colour parameters (results adjusted to fat class 3)

	Lamb genotype (L)						Diet (D)				Significance	
	B×B	SW×B	CH×B	LL×B	T×B	sed	LOW	HIGH1	HIGH2	sed	L	D
Ultimate pH	5.66	5.59	5.62	5.63	5.66	0.039	5.61	5.62	5.67	0.032	NS	NS
Cooking loss (%)	24.5	24.1	24.4	24.4	24.7	1.03	24.6	24.8	23.9	0.84	NS	NS
Sarcomere length (mm)	1.84	1.82	1.86	1.88	1.84	0.059	1.84	1.88	1.83	0.048	NS	NS
WBSF (kg/cm ²)	1.54	1.86	1.85	1.78	1.68	0.190	1.75	1.63	1.84	0.154	NS	NS
<i>Colour (illuminant D65, 2 degree observer)</i>												
L^*	41.7	41.8	41.2	37.8	40.8	2.42	43.7 ^b	41.0 ^b	37.3 ^a	1.95	NS	**
a^*	14.1	14.1	14.6	15.2	14.1	1.05	13.6	15.2	14.5	0.85	NS	NS
b^*	11.2	10.9	11.7	11.4	11.9	0.68	11.1	12.3	10.9	0.55	NS	NS ⁺
C^*	18.2	18.0	18.9	19.1	18.6	1.10	17.8	19.7	18.2	0.89	NS	NS
Hue	38.4	38.6	39.1	36.5	40.6	2.08	40.3	38.8	36.8	1.68	NS	NS

NS = $P > 0.1$, NS⁺ = $P < 0.1$, ** = $P < 0.01$, *** = $P \leq 0.001$.

L^* = lightness, a^* = redness, b^* = yellowness, C^* (metric chroma) = $(a^{*2} + b^{*2})^{0.5}$, Hue = $\tan^{-1}(b^*/a^*)$.

Conclusions Meat quality was similar for all lamb genotypes evaluated, independent of dietary regime. The concentrate diet containing oilseed rape did, however, affect meat colour, which may have implications for shelf-life in terms of appearance.

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Influence of sex type on tenderness of beef as measured by slice shear force and Warner-Bratzler shear force

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Introduction The standard instrumental method for measurement of texture, Warner Bratzler shear force (WBSF), involves shearing six 'good' cores taken from a cooked sample of meat, with coring parallel to the fibre direction (AMSA, 1995), which is a time consuming and destructive methodology. A new, more rapid method, the slice shear force (SSF) test was developed which involves measuring the shear force of a single slice per sample (Shackelford et al 1999a). Shackelford et al. (1999b) reported that SSF was strongly correlated with sensory scores for 14d aged meat. The aim of the current investigation was to study the effect of sex differences on instrumental meat quality and to identify the relationship between SSF measured at 2 days and WBSF values measured at 14 and 21 days post slaughter.

Materials and methods Samples of Longissimus dorsi (LD) were obtained from 50 bulls, 50 steers and 50 heifers at random from a commercial abattoir over a period of 6 days. Samples of LD, 125-150mm long, were obtained posterior to the 10th rib at 2 days post slaughter and a sub sample taken for measurement of ultimate pH (pH_u). A 25mm slice for SSF measurement was removed from the anterior end, weighed and a thermocouple probe inserted into the geometric centre of the steak. It was cooked in a double-plated grill to a centre temperature of 71°C, removed from grill, the maximum temperature reached was noted (~78°C) and the sample reweighed. A 10 mm-thick, 50 mm long slice was removed from each cooked steak parallel to the muscle fibers according to Shackelford et al. (1999a). This slice was sheared across the direction of the fibres and force to first shear peak noted. The remaining sample of LD was cut into two pieces and each vacuum packed and aged (2°C) up to 14 or 21 days post slaughter for measurement of WBSF. These samples were cooked in a water bath (75°C) and cooking loss determined prior to WBSF measurement on 12.7mm cores taken along the direction of the muscle fibres. The data were analysed for the main effects of sex type (pH & SSF) and aging period and their interactions (WBSF) using REML. Regression approaches were used to evaluate the relationships between SSF and WBSF.

Results SSF values measured at 2 days post slaughter were significantly lower in steers than bulls or heifers (Table 1). SSF cooking loss determined during SSF measurement was not significantly affected by sex type however WBSF cooking loss was significantly higher for bulls than for steers or heifers (Table 1). There was a statistically significant interaction between ageing and sex type ($p < 0.001$) for WBSF values (no significant difference between sex types after 14 days aging, but lower for steers than for bulls or heifers after 21 days). Regression analysis showed that there was a statistically significant relationship between SSF values measured at 2 days and WBSF measured at 14 days ($R^2 = 0.078$, $P < 0.001$) but not with WBSF measured at 21 days ($R^2 = 0.003$, NS). There was a significant relationship between endpoint temperature for SSF and SSF cooking loss ($R^2 = 0.23$, $P < 0.001$), however, end point temperature was not related to SSF values ($R^2 = 0.03$, NS). There were significant relationships between WBSF and WBSF cooking loss measured at 14 days ($R^2 = 0.02$, $P < 0.05$) and at 21 days ($R^2 = 0.04$, $P < 0.01$).

Table 1 Effect of sex type on meat quality measured by SSF at 2 days and WBSF at 14 & 21 days post slaughter.

	pHu	SSF		WBSF	
		% Cooking Loss	SSF (kg.cm ⁻²)	% Cooking Loss	WBSF (kg.cm ⁻²)
Bulls	5.51	27.0	19.4 ^a	29.7 ^a	3.3
Heifers	5.48	27.0	20.5 ^a	28.9 ^{ab}	3.3
Steers	5.48	26.4	15.6 ^b	28.2 ^b	3.2
significance	ns	ns	***	**	ns
sed	0.021	0.82	1.45	0.45	0.11
Days aging					
14 days	NA	NA	NA	28.8	3.2
21 days	NA	NA	NA	29.1	3.3
significance	NA	NA	NA	ns	ns
sed	NA	NA	NA	0.21	0.04
days.sex type	NA	NA	NA	ns	***

Conclusion Differences in instrumental measures of tenderness between the different sex types depend on the method used and the days aging when the measurement is made. Further work is required in order to use SSF at 2 days as a predictor of eating quality.

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A comparison of steers and heifers of the Charolais and Limousin breed for animal performance, carcass parameters and meat quality

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Introduction The beef herd in the UK comprises of a diverse range in suckler cow genotypes and terminal sires (Keady *et al.*, 2004^a), which results in major variability in the animals presented for slaughter each day. Recent data indicates that Charolais and Limousin progeny account for over 65% of the beef animals slaughtered in Northern Ireland (APHIS, 2008). The objective of this study was to evaluate sire breed (Charolais or Limousin) and gender (steer or heifer) on performance during the finishing period, carcass parameters and meat quality.

Materials and methods A total of 427 experimental animals from a series of studies (Keady *et al.*, 2004^b; Keady *et al.*, 2005; Keady *et al.*, 2007; and Keady *et al.*, 2008) were finished indoors on slatted accommodation on a range of diets, and slaughtered at the same meat plant over a two-year period. Animals were progeny from the suckler herd and sired by either a Charolais or Limousin bull. The animals included 289 and 138 Charolais (CH) and Limousin (LIM) progeny, and 120 and 307 heifers and steers, respectively. At slaughter carcasses were hung tender stretch. Meat quality assessment was undertaken on *longissimus dorsi* muscle as described by Keady *et al.*, (2007). All data were analysed by REML procedure using linear mixed model where study was treated as a random effect and animal breed and gender as fixed effects. Start age and initial live weight were included as co-variables.

Results The effect of breed and gender on animal performance, carcass parameters and meat quality are presented in Table 1. Feed intake and live weight gain during the finishing period was similar ($P>0.05$) for progeny of both breeds; and for steers and heifers. Limousin progeny had a higher ($P<0.001$) dressing proportion relative to Charolais progeny; whilst steers had a higher ($P<0.001$) dressing proportion relative to heifers. Charolais progeny produced leaner ($P<0.05$) carcasses than Limousin progeny; however progeny from both breeds produced carcasses with a similar ($P>0.05$) weight and conformation. Limousin progeny had larger ($P<0.01$) eye muscle areas than Charolais progeny. Steers produced heavier and leaner ($P<0.001$) carcasses relative to heifers. Charolais animals produced meat with a similar ($P>0.05$) ultimate pH and Warner Bratzler shear force to meat from Limousin progeny, however Charolais meat had a higher ($P<0.001$) cooking loss than Limousin meat. Meat from steers had a similar ($P>0.05$) ultimate pH and Warner Bratzler shear force to meat from heifers, however steer meat had a higher ($P<0.001$) cooking loss than heifer meat.

Table 1 The effect of breed and gender on animal performance, carcass parameters and meat quality

	Breed (B)			Gender (G)			Significance		
	CH	LIM	sem	Heifer	Steer	sem	B	G	B*G
<i>Animal performance</i>									
Feed intake (kg DM /d)	8.8	8.8	0.11	8.7	8.9	0.14	NS	NS	NS
Live weight gain (kg/d)	1.01	1.00	0.03	1.04	0.98	0.43	NS	NS	NS
Final live weight (kg)	606	601	3.5	593	614	4.4	NS	***	NS
<i>Carcass characteristics</i>									
Carcass weight (kg)	325	326	2.3	317	335	2.9	NS	***	NS
Dressing proportion (g/kg)	536	544	2.2	534	546	2.9	***	***	NS
Conformation score [#]	3.1	3.0	0.04	3.1	3.0	0.06	NS	NS	NS
Fat classification [†]	3.6	3.8	0.07	3.9	3.5	0.09	*	***	NS
Eye muscle area (cm ²)	68.6	71.1	1.08	70.0	69.7	1.20	**	NS	NS
<i>Meat quality</i>									
pH	5.53	5.54	0.01	5.55	5.51	0.02	NS	NS	NS
Cooking loss (g/kg)	280	272	2.8	272	279	0.36	***	*	NS
Shear force (kg/cm ²)	2.65	2.65	0.052	2.69	2.61	0.068	NS	NS	NS

[#] EUROP scale: 5, 4, 3, 2, 1 respectively; [†] EU fat classification, where 5 = fat and 1=lean.

Conclusion It is concluded that progeny of both the Charolais and Limousin breed had a similar level of performance during the finishing period. Limousin progeny had a higher dressing proportion, a higher fat cover and produced larger eye muscles than Charolais progeny. Heifers had a lower dressing proportion and a higher fat cover than steers. Meat from progeny of both breeds and from both genders had a similar toughness as assessed by Warner Bratzler shear force.

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Meat quality of beef cattle offered grass silage and legume / cereal wholecrop silage either alone or in combination with grass silage

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Introduction With increasing consumer awareness of meat quality and the relationship between dietary fat and the incidence of diseases, such as coronary heart disease, research has been undertaken to manipulate fatty acid (FA) profiles in beef. Diet has been shown to influence FA composition of meat (Scollan *et al.*, 2006). For example meat from finishing cattle offered red clover (legume) silage in comparison to grass silage had significantly increased polyunsaturated FA (PUFA) concentrations and a decreased *n*-6:*n*-3 ratio (3.28:2.30). The aim of this experiment was to assess the effect of offering lupins/triticale, vetch/barley and grass silage to continental beef finishing cattle on instrumental meat quality and FA composition.

Materials and methods Eighty continental cross steers were allocated to one of 10 dietary treatments. The five forage diets offered included grass silage, lupins/triticale silage and vetch/barley silage offered either as the sole forage or in combination to grass silage at a ratio of 70:30, on a dry matter (DM) basis, legume/cereal wholecrop: grass silage. The forages were offered *ad libitum* and supplemented with either 2 or 5 kg concentrates/head/day. An equal representation of steers from each forage treatment were slaughtered over 4 dates, ranging from 105 to 140 days on trial. Instrumental meat quality (ultimate pH, cooking loss, Warner Braztler shear force, and meat colour) and FA analysis were undertaken on the *longissimus dorsi* obtained from the fore-rib joint (between 7th to 11th rib) 7 days post mortem. Fatty acid analysis was carried out using a direct method for FA methyl ester synthesis as detailed by O'Fallon *et al.* (2007) and capillary column gas chromatography. Fatty acid concentrations were expressed as g FA/100g Total FA. Data were analysed as a 5 forage x 2 concentrate level factorial experiment with genotype, farm of origin, fat class and start weight included as covariates using GenStat REML.

Results There were no significant silage type by concentrate level interactions for FA composition or instrumental meat quality. Forage offered had no effect on saturated FA (SFA), monounsaturated FA (MUFA), omega 3 (*n*-3), omega 6 (*n*-6) and PUFA (Table 1). Vetch/barley:grass silage and lupins/triticale produced higher ($P < 0.05$) *n*-6:*n*-3 ratio than grass silage and lupins/triticale:grass silage. Increasing concentrate supplementation from 2 kg to 5 kg/head/day had no significant effect on SFA, MUFA, *n*-3, *n*-6, *n*-6:*n*-3, PUFA, PUFA:SFA, C18:2 9*cis*,11*trans*, *trans* FA (TFA) (not including CLA). Forage type had no effect on instrumental meat quality at 7 days of ageing (Table 2).

Table 1 Effect of forage type on fatty acid composition of meat from continental steers (g FA/100g Total FA)

	Forage					sed	Sig.
	GS	L/T	L/T:GS	V/B	V/B:GS		
Saturated fatty acid (SFA)	49.0	47.2	49.2	49.9	47.7	1.21	NS
Monounsaturated fatty acid	40.9	42.1	40.9	40.7	39.8	0.30	NS
<i>n</i> -3	1.56	1.55	1.53	1.45	1.96	0.267	NS
<i>n</i> -6	4.45	5.17	4.22	4.27	6.48	0.943	NS
<i>n</i> -6: <i>n</i> -3	2.76 ^a	3.31 ^b	2.76 ^a	2.93 ^{ab}	3.35 ^b	0.223	*
Polyunsaturated fatty acid (PUFA)	6.42	7.13	6.15	6.03	8.98	1.284	NS
PUFA:SFA	0.141	0.160	0.132	0.129	0.194	0.0289	NS
C18:2 9 <i>cis</i> ,11 <i>t</i>	0.292	0.297	0.284	0.288	0.235	0.0910	NS
<i>trans</i> fatty acids	3.30	3.18	3.31	2.97	3.16	0.527	NS

Table 2 Effect of forage type on instrumental meat quality of continental steers

	Forage					sed	Sig.
	GS	L/T	L/T:GS	V/B	V/B:GS		
Ultimate pH	5.54	5.52	5.54	5.56	5.62	0.043	NS
Cooking loss (g/kg)	312	308	301	299	300	7.9	NS
WBSF (kg/cm ²)	3.10	2.96	3.05	2.99	3.20	0.164	NS
Meat colour							
L* (lightness)	34.1	33.0	30.9	33.2	34.4	1.96	NS
a* (redness)	19.7	20.2	19.9	19.3	19.5	1.17	NS
b* (yellowness)	14.4	12.9	13.6	13.2	13.5	0.90	NS

GS: Grass silage; L/T:GS: Lupins/triticale : GS on a 70:30 DM ratio; L/T : Lupins/triticale; V/B : Vetch/Barley; V/B:GS: Vetch/Barley : GS on a 70:30 DM ratio. WBSF: Warner Bratzler Shear force.

Conclusion Offering finishing beef cattle legume/cereal wholecrop silages had no effect on fatty acid composition or meat quality relative to grass silage.

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The effects of supplementation of maize silage diets during pregnancy on ewe and subsequent lamb performance

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Introduction Maize silage can be produced at a similar cost to grazed grass and is approximately 20% cheaper than grass silage (Keady *et al.*, 2002). Recent studies have shown that maize silage inclusion in the diet increases the performance of dairy (Keady *et al.*, 2003, 2008) and beef (Keady and Gordon, 2006, Keady *et al.*, 2007) cattle and has a concentrate sparing effect of up to 2.5 kg and 5.0 kg, respectively (Keady, 2005). Previous studies at Athenry have shown that maize silage, regardless of maturity at harvest, results in similar levels of ewe and subsequent lamb performance relative to high feed-value grass silage, (Keady and Hanrahan, 2008, 2009). The objective of the current study was to evaluate the effects level and type of concentrate supplementation of maize silage diets on ewe and subsequent lamb performance.

Materials and methods Maize which was sown on 5 April and grown under the complete plastic mulch system was harvested on 8 October and ensiled treated with a bacterial inoculant. A total of 36 ewes [(Belclare x Scottish Blackface, Chamoise x Scottish Blackface) (initial live weight = 69 kg, initial condition score = 3.7)] were offered maize silage *ad libitum* from mid pregnancy to lambing and were allocated at random to three concentrate treatments: 200 g soya bean daily during mid and late pregnancy plus 15 kg concentrate in late pregnancy (SC), 10 kg soya bean during late pregnancy (10S) and 5 kg soya bean during late pregnancy (5S). The ewes were moved to pasture within 3 days of lambing. Ewes rearing singles or twins, and their lambs, were offered no concentrate at pasture. Ewes rearing triplets received 0.5 kg concentrate daily for the first 5 weeks post lambing, whilst their lambs were offered up to 300 g concentrate daily until weaning at 14 weeks. The data was analysed using Proc GLM and Proc MIXED of SAS as appropriate.

Results The mean dry matter (DM), crude protein, starch and ME concentrations of the maize silage were 339 g/kg, 88 g/kg DM, 236 g/kg DM and 11.5 MJ/kg DM, respectively. The effects of concentrate supplementation on ewe and subsequent lamb performance are presented in Table 1. Ewes offered 5S had significantly lower ($P<0.001$) condition at lambing. Otherwise level or type of concentrate supplementation did not alter ($P>0.05$) ewe or subsequent lamb performance.

Table 1 Effect of supplementation of maize silage on ewe and subsequent lamb performance

	Treatment			s.e.	sig
	SC*	10S	5S		
Ewe weight (kg) - lambing	74.9	72.0	66.5	2.95	NS
- weaning	68.2	69.3	66.4	2.55	NS
Ewe condition score at lambing	3.60	3.57	2.84	0.142	***
Litter size	2.05	2.07	2.00	0.198	NS
Number reared/ewe lambing	1.58	1.79	1.46	0.226	NS
Lamb weight - birth	4.7	4.8	4.4	0.22	NS
- weaning	30.4	30.9	29.7	2.05	NS
Lamb gain (g/d) - 0 to 5 weeks	280	274	241	25.0	NS
- birth to weaning	262	270	261	20.4	NS

*SC = 200g soya bean meal daily and 15 kg concentrate in late pregnancy, 10S = 10 kg soya bean meal in late pregnancy, 5S = 5 kg soya bean meal in late pregnancy

Conclusions There is no benefit to supplementing ewes offered maize silage with protein in mid pregnancy. During late pregnancy concentrate supplementation can be reduced to approximately 10 kg of soyabean meal without having a negative impact on ewe or subsequent lamb performance.

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The effects of incorporating either lupins or soya bean meal into concentrate diets when compared with a control concentrate diet on the performance and carcass characteristics of finishing lambs

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Introduction Soya bean (*Glycine max*) meal is an important component of animal feed and oil seed rape and palm kernel cake and meal are typically used in commercial concentrate diets for sheep in the UK. Lupins (*Lupinus*; *Leguminosae*) as a high protein, high energy, nitrogen-fixing grain legume, have potential as a home-grown livestock feed in the UK (Wilkins and Jones, 2000). Research has been conducted on the effects of narrow-leaved lupins (*Lupinus angustifolius*) (Hill, 2005) but there have been few studies on the effects of yellow lupins (*Lupinus luteus*) when fed to sheep. This study investigated the effects of incorporating either yellow lupins, narrow-leaf lupins or soya bean meal into the concentrate diets of finishing lambs on lamb productivity and carcass characteristics when compared to a commercial UK lamb finisher diet.

Materials and methods The experiment comprised of a lamb finishing experiment, using eighty male castrated Suffolk-cross lambs to give with 20 lambs on each of 4 dietary treatments. Treatments were narrow-leaved lupin (cv. Prima), yellow lupin (cv. Wodjil), soya bean meal and a commercial (control) lamb finisher diet. The concentrate diets (pelleted) had a target nitrogen concentration of 25.7 g/kg DM (16 % CP) and ME of 11.0 MJ/kg DM. The two lupin varieties for the experiment were produced on the same experimental site (at IBERS) and harvested according to standard practice. The experiment consisted of 3 phases: a 14-d covariate, a 14-d adaptation and an 8-week measurement period (Day 0 - 56). During the covariate period, lambs were kept as one group on a ryegrass /clover sward. At the start of the adaptation period, lambs were allocated to their replicate blocking group within each treatment on the basis of live weight and body condition score. Prior to the measurement period, lambs were housed and dietary treatments were gradually introduced, along with straw as the fibre component of the diet. Each blocking group was allocated at random to a set of adjacent pens (n = 5 per pen) and treatments allocated at random to pens within this. During the measurement period, animals were offered straw and supplemental concentrate *ad libitum*, with a refusal margin of 0.10 to 0.15 d⁻¹. Concentrate as offered was DM sampled and sub-sampled daily and bulked weekly, freeze dried and ground for chemical analysis. Individual lambs were weighed and condition scored every 7-d throughout the experiment. The measurement period was divided into two phases. During week 1 to 4, liveweight gain data from all lambs were recorded. From week 5 onwards, lambs were selected-out for slaughter as they reach a target fat class of 3L and live weight and cold carcass weight were used to determine killing-out percentages. Daily liveweight gain data were compared using a non-parametric regression of Theil (1950), as implemented by Dhanoa (1998).

Results The DM content of the concentrate diets were 859, 850, 867 and 874 g kg⁻¹ freshweight for the soya bean meal, narrow leaf lupin, yellow lupin and control diet, respectively. The N concentration of the soya bean meal, narrow-leaf lupin, yellow lupin and control diet was 32.2, 32.9, 33.4 and 30.8 g kg⁻¹ DM, respectively. The ME concentration of the soya bean meal, narrow-leaf lupin, yellow lupin and control diet was 12.5, 12.8, 12.6 and 12.6 MJ/kg DM, respectively. There was no significant effect ($P > 0.05$) of dietary treatment on the liveweight gain of lambs offered the different diets. The liveweight gain of lambs offered concentrates incorporating soya, narrow-leaf lupin, yellow lupin or a commercial control diet were 185, 229, 193 and 166 g/d, respectively (s.e.d. 34.6) The carcass characteristics of the lambs are given in Table 1.

Table 1 Carcass characteristics (mean $\pm$ se) and number of days to finish of lambs offered concentrates containing either soya bean meal, narrow-leaf lupins, yellow lupins or commercial control finisher diet *ad libitum*.

	Soya	Narrow-leaf lupin	Yellow lupin	Control
Empty liveweight (kg)	38.5 $\pm$ 0.77	38.3 $\pm$ 0.52	38.5 $\pm$ 0.70	37.6 $\pm$ 0.61
Hot carcass weight (kg)	19.3 $\pm$ 0.39	19.4 $\pm$ 0.32	19.8 $\pm$ 0.40	18.8 $\pm$ 0.35
Killing-out %	50.1 $\pm$ 0.34	50.7 $\pm$ 0.37	51.3 $\pm$ 0.31	50.1 $\pm$ 0.32
Days to finish	33 $\pm$ 2.1	30 $\pm$ 0.5	32 $\pm$ 2.0	32 $\pm$ 1.8

Conclusion Overall, the findings from this study show that both narrow-leaf and yellow lupins could be used as a home-grown alternative to imported soya or a bought-in commercial concentrate, containing rapeseed meal and palm kernel, for finishing lambs in the UK without any adverse effects on lamb productivity or carcass killing-out percentages.

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The effect of alpha-tocopherol concentration and antioxidant enzyme activities on the shelf life stability of beef from different feeding systems

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Introduction The oxidative stability of meat depends upon a combination of pro- and antioxidants and the degree of unsaturation of its lipid classes. When animals graze grass the concentrations of α -tocopherol and β -carotene in meat and fat increase and decrease when they are fed concentrates. Supplementation with α -tocopherol acetate increased α -tocopherol concentration in the meat of concentrate-fed animals, less so for grass-grazed animals (Descalzo et al., 2005; Realini et al., 2004). Less is known about the relationship between diet, antioxidant enzymes systems such as Superoxide dismutase (SOD) and Catalase (CAT), and the oxidative stability of meat. This study aimed to determine the influence of a forage versus a concentrate diet fed to beef cattle, and an additional treatment with a PUFA-rich lucerne-based plant extract (PX), on the antioxidant profile of their meat, and its relationship with lipid oxidation and colour shelf life. The addition of PX raised the unsaturated nature of the beef muscle lipids and thus increased the oxidative stress (Kim et al., 2009).

Material and methods 29 Charolais x Friesian steers were allocated, based on live weight and age, to one of 3 dietary treatments; G1: 9 steers fed forage (grass/white clover swards during grazing followed by grass silage in winter period), G2: 10 steers, fed restricted barley straw and concentrate (40:60 on a DM basis), and G3: 10 steers, fed the restricted barley straw and concentrate with PX. Liveweight was monitored every 28 days and gains were calculated for individual animals. This data was used to modify feed intake of the G2 and G3 animals to achieve a similar growth rate to those fed on *ad libitum* forage. Animals were slaughtered, the carcass chilled for 48h and *M. longissimus thoracis* sampled. Tocopherol analysis was performed by the methodology of Buttriss and Diplock (1984). Colour (L^* , a^* and b^*) was measured daily on 10 day aged steaks kept under simulated retail display (MAP, O₂:CO₂, 75:25, 700 lux, 16h light, 8h dark, 4°C) and Chroma calculated ($C^* = \sqrt{(a^{*2} + b^{*2})}$). Thiobarbituric acid reacting substances (TBARS) were determined on a steak after 10 d in retail display (Tarladgis et al., 1960), SOD and CAT activities were determined spectrophotometrically (Marklund and Marklund, 1974; Aebi, 1974, respectively). Data for TBARS was logged before statistical analysis. Analysis of variance and Pearson correlation were carried out in SPSS v14.0

Results The concentration of α -tocopherol was highest in the meat of grass-grazed animals (G1) and lowest in the animals fed concentrate (G2 and G3) and this affected TBARS, which were low in G1 but highest for G2 and G3 (Table 1). The change in Chroma (ΔC^*) between day 1 and day 10 was greatest for the least stable meat (G2 and G3). SOD activity in meat was not influenced by diet (data not shown). Catalase activity was highest in the meat of those animals fed concentrates (G2 and G3). Negative correlations ($P < 0.01$) were found between α -tocopherol content, TBARS and the change in C^* after 10 days storage in MAP (Table 2). SOD activity also showed negative correlations but these were not statistically significant. Catalase activity was positively correlated with change in colour.

Table 1 Effect of diet on antioxidants, colour and lipid oxidation in beef loin muscle.

	ΔC^* 10days	α -Toc mg/g	TBARS mg/g	CAT unit/mg protein
G1	7.83 ^a	5.08 ^a	1.1 ^a	9.50 ^a
G2	11.77 ^b	2.35 ^b	3.4 ^b	13.43 ^b
G3	12.32 ^b	3.01 ^c	5.7 ^b	12.64 ^{ab}
sig	***	***	***	***

^{abc} values with different superscripts are statistically significantly different (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$)

Table 2 Pearson correlations between α -tocopherol, antioxidant enzymes, colour change and TBARS in beef

	SOD	CAT	ΔC^*	TBARS
α-toc	0.14	-0.39*	-0.43**	-0.59**
SOD		-0.11	-0.17	-0.22
CAT			0.44*	0.25
ΔC^*				0.75**

Conclusions Meat from grazed cattle had an improved colour and lipid stability than that from concentrate-fed cattle due, to a higher deposition of α -tocopherol. Whilst the antioxidant enzyme, SOD, did not appear to be affected by diet, or correlated with measures of oxidation, catalase activity increased when α -tocopherol concentration was low and consequently lipid oxidation was higher.

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Forage based fattening of male buffalo calves (*Bubalus bubalis*) under farmers' managed condition in Dang district, Nepal

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Introduction Buffaloes (*Bubalus bubalis*) are an important component of livestock in Nepal. Its population has been estimated as 4,204,886 in 2005/06 (MOAC, 2006). Buffalo calves are seldom raised for meat in Nepal (Rana *et al.*, 2000). In cases where they are raised, they are confined until six to eight months of age, as their dams will not give milk without them (Rana *et al.*, 2000). Scientific information on fattening of male buffalo calves is scanty. A field based study was conducted to access the body weight of male buffalo calves raised under forage based and forage based plus limited concentrate supply at different ages, to determine the cost effectiveness of fattening technology under farmers' managed condition.

Materials and methods The study was conducted from May to November 2006, in Saudiyar, Dang, Nepal. Twenty indigenous male buffalo calves, aged about 8 months were identified, and raised as one calf from each household. Calves were treated against parasites and common diseases. A randomized complete block design (RCBD) with five replications was employed with 2×2 factorial combination of treatments. The two feeding management regimes were forage only (F), and forage plus limited (about 7% of dry matter requirements) concentrate feeding (FC); Calves in both regimes were raised for either 90 or 180 days of fattening. Weighing was done at 30 d intervals. A farm recorder was employed to keep necessary information. Analysis of Variance (ANOVA) was done using GenStat Version 5.4.2. Least Significance Difference (LSD) was used to compare the means using $\alpha = 0.05$. Covariate analysis was done using initial body weight as a covariate. Total variable cost for each treatment was calculated, the fixed cost and miscellaneous expenses were kept constant. Dressing percentage was taken as 60% of the gross body weight. Return from animals was calculated considering the prevailing market price in the locality.

Results The initial mean body weight of male buffalo calves (aged ~8 months) was 92.50 kg, which then weighed 123.70 kg and 153.30 kg, after 90 and 180 days fattening, respectively. There was a significant difference ($P < 0.05$) between treatments for mean daily live weight gain (MDWG) with the highest value for FC, fattened for 180 days (414 g) while the lowest gain was obtained for forage based only, fattened for 90 days (271 g). Feeding management had a significant effect ($P < 0.05$) on the MDWG, whereas effect of fattening duration for MDWG was similar ($P > 0.05$). The adjusted MDWG values also reflected similar trends. There was no significant interaction between feeding management and fattening period (Table 1).

Table 1 The mean daily weight gain (g) of male buffalo calves, Saudiyar, Dang, Nepal, 2006.

Feeding management (A)	MDWG (g)		Adjusted MDWG (g)*	
	F	FC	F	FC
	277.00	405.00	289.00	394.00
Fattening period (B)	90 D	180 D	90 D	180 D
	334.00	349.00	343.00	340.00
T1 (Forage based fattened for 90 days)	271.00 ^c		281.00 ^c	
T2 (Forage based fattened for 180 days)	284.00 ^{bc}		298.00 ^{bc}	
T3 (Forage; conc.; fattened for 90 days)	397.00 ^{ab}		405.00 ^a	
T4 (Forage ;conc.; fattened for 180 days)	414.00 ^a		382.00 ^{ab}	
Treatments	P<0.05 (117.9)		P<0.05 (97.1)	
Feeding management (A)	P<0.05 (83.4)		P<0.05 (69.1)	
Fattening period (B)	NS		NS	
A×B	NS		NS	

*Based on covariate analysis, by following initial weight as covariate; figures in parentheses are LSD value

The cost per kg dressed meat production for both F treatment and FC was slightly higher when fattened for 180 days (NRs 53.80 and 52.10, respectively) than when fattened for 90 days (NRs 50.81 and 48.04, respectively), whereas the profit per kg dressed meat production for F and FC, respectively, was slightly higher for 90 days fattening (NRs 33.66 and 35.11, respectively) compared with 180 days fattening (NRs 30.99 and 30.48, respectively)

Conclusion These results show that short duration fattening (90 days after 8 months) of male buffalo calves based on forage and limited feed supply could be the better option for higher profit per kg meat. Further research is, however, required before recommending appropriate option for wider scale of rearing.

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Evaluation of genetic trends for milk yield and somatic cell counts in the Holstein breed in terms of changes in the lactation curve

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Introduction The use of test day model (TDM) for genetic evaluation results in predicted transmitting abilities (PTAs) for any stage of lactation. However, in the United Kingdom (UK), publication and selection of parents are still based on PTAs computed on a 305-day basis from the random regression coefficients of the TDM. It is expected from selection index theory, that selection on 305-day PTAs would place different weights on PTAs at different days in milk (DIM). This could result in changes in the lactation curve of animals over time as a result of genetic progress. This study evaluates genetic trends in terms of changes in PTAs at different DIM for production traits and somatic cell counts (SCC) in the Holstein breed. In addition, the inclusion of SCC in the national profitable life index (£PLI) is based on its relationship with milk computed on a 305-day basis. This study also examines what impact such selection has on the relationship between PTAs for milk and SCC at different DIM.

Materials and methods PTAs for 305-day and 10 stages of lactation for bulls and cows were computed from the first parity random regression coefficients of the official August 2008 TDM evaluation for the Holstein breed for milk in the UK. Details of the TDM have been described by Mrode et al 2005. Similarly, a TDM for first parity SCC was implemented fitting Legendre polynomial of order 1 and 2 for animal effect and permanent environment (Mrode and Swanson, 2003). The analysis for production traits involved 6,450,558 cows born from 1974 to 2006 and with about 58 million test day (TD) records. A total of 3,294,634 heifers born 1986 to 2006 with 27,895,096 TD records were evaluated for SCC. Genetic trends were estimated for 305-day and different DIM by regressing PTAs on the year of birth. The percentage contribution of the trend at each DIM to the overall trend was computed. The average genetic lactation curve was examined over time. Correlations of PTAs for milk and SCC for bulls with at least 50 daughters for both traits were computed at 305-day basis and different DIM; then changes in the relationship between both traits over DIM were examined.

Results Bull genetic trends for first parity milk 36.118kg per year over the period 1964 to 2003. The rate of change for PTAs at 120 – 210 DIM accounted for 43% of the trend observed on the 305-day basis, with changes for PTAs at 30 DIM accounting only for about 7-8%.

In general the highest rate of change (11%) was observed for PTAs at 150 and 180 DIM. Figure 1 illustrates that genetic progress has resulted in the shift of the peak of the curve from day 60 in 1964 to 150 in 2003. This shift was similarly observed when analysis was done within bulls originating from countries with mainly pure Holstein populations, so it is not due only to changes in breed composition. The cow genetic trend for milk was 38.465kg/year on 305-day basis, with about 11% of the change observed at 150 DIM. The plot of the genetic curve for cows followed the same pattern as bulls. In contrast, the rate of change for SCC was linear with DIM in milk, with +36, +1 and -22% of the rate for 305-day basis contributed by SCC PTAs at 30, 210 and 305 DIM. The genetic curve for SCC indicates that this linear increase has declined with time. It seems that the reduction in SCC as a

result of selection has mainly been due to reduction in SCC at mid to later stages of lactation. This is consistent with estimates of correlation between Bull PTAs for milk and SCC. While the correlation of 305-day milk and SCC PTAs was 0.22, the estimate between 305-day milk PTA and SCC PTAs at 30, 150, 270 and 305 DIM were 0.41, 0.30, 0.06 and 0.01. This indicates a stronger antagonistic relationship between milk and SCC at early stages of lactation.

Conclusion The rate of change of milk PTAs at various DIM was greatest at about mid lactation. Thus genetic progress has been accompanied by a shift in the peak of the genetic lactation curve. While there has been a decrease in SCC at late lactation, rate of SCC seems to have increased in early lactation.

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Selection for milk production in a single Holstein herd: effects on correlated traits

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Introduction Selection is the main tool used by animal breeders to improve the performance of their stock. Despite large gains being made at industry level in the major dairy traits (Royal *et al.*, 2000) very few reports exist for the results of experiments on persistent selection for dairy traits in a single herd, particularly with reference to the correlated effects on other dairy traits. This paper reports one such set of results between 1980 and 2005 from SAC's Langhill herd, which has been under maximal selection for milk production during this period.

Materials and methods This work was based on the intensively recorded Langhill herd of cows originally maintained at The University of Edinburgh's Langhill Farm until 2002, and then transferred to SAC's Crichton Royal Farm. Details of the management of this herd have been described by Pollott and Coffey (2008). This 200-cow herd was equally divided into a Selection Line and a Control Line; only results from Selection Line animals are reported in this paper. Bulls were chosen from all AI bulls available at the time of mating which had the highest fat plus protein PTAs, had semen available and were not related to the cows back to grandparent level. The data used in this study were the PTAs for all traits produced as part of the national genetic evaluations carried out by EGENES on behalf of DairyCo. Results presented are the mean annual PTA values for Langhill Selection Line cows each year between 1980 and 2004. Linear regression on year of birth was applied to each set of trait PTAs to estimate the mean annual genetic response.

Table 1 The genetic gain (1980-2004), change per year, its standard error and the fit for the linear regression of trait on year of birth for cow PTAs

Trait	Genetic gain	Change per year	SE	R ²
Fat+protein (kg)	76.2	2.66	0.106	0.576
Milk (kg)	717.5	34.9	2.13	0.365
Test-day yield (kg)	7.56	0.295	0.0121	0.563
Persistency (%)	2.13	-0.859	0.0144	0.071
Calving Interval (d)	22.7	0.671	0.0351	0.438
Non-return rate	-0.057	-0.0032	0.000228	0.292
Number of services	0.128	0.00663	0.000500	0.271
Days to 1 st service	6.08	0.418	0.0217	0.445
Condition score	-2.11	-0.0672	0.00311	0.498

Results The genetic trend information summarised in Table 1 was derived from 466 cows born in the Selection Line from 1980 to 2004. Selecting sires on the basis of their fat+protein PTA resulted in an average annual PTA increase of 2.66kg per lactation. The other changes shown in Table 1 represent correlated genetic responses in other traits occurring because of the selection on fat+protein PTA. Milk production increased by 35kg a year and test-day yield at about day 110 also increased. All other traits deteriorated with cows having poorer fertility, persistency and becoming thinner. The regression of each trait PTA on year of birth explained a differing amount of the variation in each trait. In several traits the regression line accounted for more than half of the variation whereas the regression of persistency PTA on year had a poor fit to the data. Over the 14-year period described by these results the proportion of Holstein genes in the Selection Line cows rose from about 0.25 to almost 1.0.

Conclusions Several authors have reported on the changes that have occurred in dairy cow productivity and fertility at national or regional level (Butler and Smith, 1989; Royal *et al.*, 2000; A.-Ranberg *et al.*, 2003). The general trend is for an improvement in milk production at the expense of fertility. However, such studies do not account for the effects of farm, management, feeding changes and health status on the measured traits. In this study we have demonstrated that selection for increased milk production unequivocally leads to deterioration in genetic merit for fertility and condition score.

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An investigation on the effect of management factors and contemporary group definition on the genetic evaluation of Calving Interval in British Limousin cattle

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Introduction Female fertility is an important component of a beef production system. In the British Limousin genetic evaluation there are several female fertility traits recorded (Coffey *et al.* 2007). Two of these traits are calving interval (CI) and age at first calf (AFC). In British beef breeds, Roughsedge *et al.* (2005) estimated moderate negative genetic correlations between CI and AFC; early maturing animals (low AFC) had longer CI because of genetic factors. However, it has also been suggested that management factors could be increasing the CI EBV of early maturing animals. For example, does the CI EBV account for heifer's that calve early for the first calf, but then are not given the opportunity to re-mate when they first return to oestrus, and therefore, may have a longer raw CI record. There were two aims of this work 1) to investigate if CI EBVs produced by the British Limousin genetic evaluation were affected by management decisions applied to early maturing animals (i.e. low AFC) and 2) to assess if reducing the season slice (i.e. reducing the chance of directly comparing early and late calvers) for the CI contemporary group (CG) improved the CI model.

Materials and methods CI was defined as the number of days between a heifers first and second recorded calf. EBVs were produced using the software Mix99 (Lidauer and Strandén, 1999) that is based on Best Linear Unbiased Prediction. The model consisted of the CG, birth month of the first calf and the Limousin breed proportion as fixed effects, the linear and quadratic effect of the raw AFC and the heifer fitted as a random effect. The CI CG was defined by the herd, year and season of the first calf's birth, with season being a six month time period. CI records were considered to be invalid if the CI interval was less than 290 days or greater than 630 days, or if the animal was in a single record CI CG. In this study two models were considered. The first is described above and was used for the Limousin British genetic evaluation. The second model was the same as the first, except that the season slice in the CG was reduced from six to three months.

Results Decreasing the CI CG season slice to three months reduced the number of animals per CG from three to two, and increased the number of single record CGs. This meant that the number of animals with CI information differed between the models. There were 23 073 and 19 814 animals with valid CI and AFC records when the season slice was six and three months, respectively. In both data sets, the heifers average raw AFC was 992 days (range=548 to 1278 days) and the raw CI records were similar at 403 and 401 days, respectively when the season slice was six and three months.

Figure 1 The relationship between calving interval (CI) and age at first calf (AFC) EBVs when season length in the CI contemporary group definition varied. Data points represent 10 percent of the population ranked on the AFC EBV

When the top and bottom 10% of animals based on the raw AFC were compared, a difference of 2.12 days ($P < 0.01$) and 4 days ($p = 0.04$) was observed for the CI EBV and raw value, respectively. These differences were not unexpected given the moderate negative genetic correlation. However, the difference in the raw CI was not large enough to suggest that early calvers across the population were being penalised by management decisions - such as delayed re-mating - that may affect CI.

It can be seen in Figure 1 that reducing the season slice did reduce the CI EBVs. However, this reduction was uniform across the population and there was no relationship with the AFC EBVs.

Conclusions These results suggest that across the British Limousin cattle population, any bias against early maturing heifers from management decisions did not adversely affect the CI EBVs. Within individual herds and CG, there may be bias against early calvers. However, with limited information recorded it is difficult to identify these CG and account for differences in mating appropriately. With the small CG sizes despite a broad CG definition, this study highlighted the small amount of information available for maternal traits. As more information is recorded, reducing the length of the season slice may improve the CI EBVs but in this study, reducing the season length did not appear to improve the CI EBVs.

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Preliminary genetic analysis of beef carcass data

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Introduction Selective breeding of farm livestock is one of the most cost-effective ways of improving the performance and efficiency of livestock enterprises. Genetic improvement of British beef cattle over a ten year period was recently estimated to be worth approximately £23, and the benefits continue to rise (Amer *et al.*, 2007). While these returns are impressive, they could be improved by increasing the rate of improvement in the purebred population, for example by increasing the relevance of estimated breeding values (EBVs) to beef production by using final carcass weight and grading information. This study will examine the feasibility carcass weights and classifications from UK commercial abattoirs for the genetic evaluation of cattle for carcass weight, carcass fatness class, and carcass conformation class.

Materials and methods This study used data from Dovecote Park, which supplies beef to Waitrose supermarket chain. Extensive data is recorded on the animals after slaughter. Carcasses were classified at the abattoir using the EU beef classification scheme for conformation (EUROP) and fat class (1-5). The carcass traits analysed in this study included carcass weight (CWT, kg), adjusted carcass conformation score (CCON), and adjusted carcass fat score (CFAT), the latter two adjusted to a 15 point scale. The identity of the animal was linked to a sire via British Cattle Movement Survey data and data were edited to only include records with a valid sire identity. A final edit required each sire to have a minimum of 2 offspring with valid data and last herd (i.e., finishing herd) was required to have a minimum of 2 observations. This resulted in a final dataset of 14923 records pertaining to 1449 sires. Variance components were estimated in a multiple trait model for CWT, CCON and CFAT by REML based on a sire model using ASReml (Gilmour *et al.*, 2006). Relevant significant model effects were fitted, with non significant components dropped. For all 3 traits breed of dam, age at slaughter (linear and quadratic terms), sex of animal and interaction of last herd with year were fitted in the model with sire identity fitted as a random effect. Additional effects fitted included season and year of birth for in the models for CCON and CFAT.

Results Figure 1 shows the distribution of phenotypic records for CFAT and CCON, with a skewed and narrow distribution in both traits. Animals were, on average, 24.8 months of age at slaughter with an average CWT of 305.6 kg (164.4 – 446.5 kg). Table 1 gives the genetic parameters for the three carcass traits, CWT, CCON and CFAT. Carcass classifications (CCON and CFAT) have a moderate heritability, with a moderate correlation between the two traits. Carcass weight resulted in a high heritability (0.85), which is generally higher than in other reported studies (Ríos Utera and Van Vleck, 2004). However, it

should be noted that this analysis does not fit the genetic relationships between sires in estimating the genetic components. Also, heterosis is likely to have an effect on the carcass traits of crossbred animals, which is not considered in this study. The results indicate that there is considerable genetic variation among sires for the studied traits. For example, the carcass of offspring of the genetically top 5 sires for carcass weight is expected to be, on average, 72.9 kg heavier than the carcass of offspring of the worst 5 sires for this trait; this is equivalent to 24% of the mean carcass weight.

Conclusions The results of this study provide a strong indication of the existence of genetic variation in carcass traits of UK beef animals and therefore improving beef carcass quality traits through genetic selection is possible. The development of routine EBVs for carcass traits will be enhanced with increased capture of accurate abattoir carcass data and more detailed analysis of the genetic parameters.

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Figure 1 Distribution of carcass conformation score and carcass fat score in the data (E+ is best conformation and P- is worst; 1- is leanest and 5+ is fattest)

Table 1 Genetic parameters for carcass traits, CCON, CFAT and CWT. Heritabilities on the diagonal, genetic correlations above diagonal and residual correlations below diagonal (standard errors in brackets).

	CCON	CFAT	CWT
CCON (1-15 scale)	0.39 (0.035)	0.40 (0.071)	-0.02 (0.059)
CFAT (1-15 scale)	0.18 (0.009)	0.23 (0.029)	0.14 (0.066)
CWT (kg)	0.27 (0.008)	0.21 (0.009)	0.89 (0.049)

Genetic correlations between piglet birth weight, its variation and survival in different dam and sire lines using Bayesian analysis

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Introduction In the UK, mean stillbirth rate is reported to be 6.7% (BPEX, 2008), which indicates a considerable loss for pig producers. Lately much research has been carried out to improve piglet survival by genetic improvement, but correlations between survival and reproduction traits showed contradictory results and seldom distinguished between maternal and direct piglet genetic effects. Some studies considered birth weight as a trait that is likely to be closely related to survival, but few looked at individual piglet birth weight. The aim of this study was to estimate genetic correlations between birth weight or its variation within litter and survival to examine if selection on individual birth weight is a viable way to improve survival.

Materials and methods Data for over 36,000 piglets born in 2005 and 2006 from three dam lines (Landrace (LR-D), Large White (LW-D) and synthetic (S-D)) as well as two sire lines (Large White (LW-S) and synthetic (S-S)) from the pig breeding company PIC were analysed. The dam lines focussed on litter size and survival, with added emphasis on growth rate in the S-D line. The LW-S line focussed on body leanness, while the S-S line focussed on growth rate, feed efficiency and growing pig survival. The traits included percentage piglet survival at birth per litter (SVBL), individual piglet survival at birth (SVBP), individual birth weight (BW), average birth weight per litter (ABWL) and standard deviation of birth weight within litter (STDBW). Fixed effects included parity, gestation length, batch and sex. Multiple trait analyses were carried out in a Bayesian approach, using THRGIBBS1F90 and GIBBS2F90 (Misztal et al., 2002), to account for the threshold character of individual piglet survival and to obtain Bayesian confidence intervals indicating the precision of the estimates.

Results Table 1 shows that heritabilities for survival at litter level were generally low, though the LW lines yielded a slightly higher heritability than the other lines. These LW lines had the lowest average birth weight (LW-D, 1385 g; LR-D, 1277 g), whereas the other lines had weights in the range of 1442 to 1589 g. Birth weight showed clearly higher heritabilities than survival. Genetic correlations of SVBL with ABWL and STDBW were moderately favourable except for SVBL-ABWL in the LR-D line and SVBL-STDBW in the S-S line. At piglet level, as presented in Table 2, the LW-D line showed negative correlations between survival and birth weight, whereas the LR-D and S-S lines displayed desirable positive correlations.

Table 1 Heritabilities (h^2) of SVBL, ABWL and STDBW and genetic correlations (r_g) of SVBL with ABWL and STDBW for three dam lines (LR-D, LW-D and S-D) and two sire lines (LW-S and S-S) (95%-HPD as subscript)

	h^2_{SVBL}	h^2_{ABWL}	h^2_{STDBW}	$r_g_{SVBL-ABWL}$	$r_g_{SVBL-STDBW}$
LR-D	0.05 _{0.01 - 0.12}	0.23 _{0.12 - 0.35}	0.11 _{0.04 - 0.18}	-0.50 _{-1.00 - 0.18}	-0.52 _{-1.00 - 0.27}
LW-D	0.13 _{0.03 - 0.23}	0.30 _{0.20 - 0.41}	0.10 _{0.03 - 0.17}	0.35 _{-0.14 - 0.90}	-0.18 _{-0.86 - 0.80}
S-D	0.08 _{0.00 - 0.17}	0.26 _{0.09 - 0.42}	0.10 _{0.02 - 0.19}	0.55 _{-0.09 - 0.99}	-0.18 _{-0.99 - 0.60}
LW-S	0.20 _{0.01 - 0.38}	0.26 _{0.08 - 0.45}	0.27 _{0.10 - 0.46}	0.47 _{-0.25 - 1.00}	-0.43 _{-1.00 - 0.34}
S-S	0.08 _{0.00 - 0.18}	0.34 _{0.21 - 0.48}	0.14 _{0.05 - 0.26}	0.22 _{-0.43 - 0.86}	0.48 _{-0.33 - 1.00}

Table 2 Direct (h^2_d) and maternal (h^2_m) heritabilities of SVBP and BW and genetic correlations (r_g) between the direct and maternal effects of both traits in the three dam lines (95%-HPD as subscript)

	h^2_{dSVBP}	h^2_{mSVBP}	h^2_{dBW}	h^2_{mBW}	$r_g_{dSVBP-dBW}$	$r_g_{mSVBP-mBW}$
LR-D	0.01 _{0.00 - 0.01}	0.06 _{0.02 - 0.10}	0.13 _{0.04 - 0.25}	0.28 _{0.16 - 0.39}	0.62 _{0.23 - 0.94}	0.18 _{-0.21 - 0.58}
LW-D	0.01 _{0.00 - 0.01}	0.07 _{0.03 - 0.10}	0.19 _{0.09 - 0.30}	0.16 _{0.08 - 0.25}	-0.42 _{-0.83 - -0.05}	-0.17 _{-0.55 - 0.25}
S-D	0.03 _{0.00 - 0.07}	0.08 _{0.04 - 0.14}	0.18 _{0.03 - 0.37}	0.26 _{0.12 - 0.40}	0.67 _{0.15 - 1.00}	0.72 _{0.47 - 0.94}

Conclusions All lines showed considerable variation for survival and relatively high heritabilities for this trait in lines with low average birth weight. Maternal heritabilities of birth weight were mostly at moderate magnitude and thus of great interest for selection. For most lines, the correlations between traits indicate that selection on either individual or average birth weight seems feasible to increase survival at birth. Interestingly, in the LR-D line the correlation between survival and birth weight was unfavourable at litter level but favourable at piglet level. This can be explained by higher importance of variation of birth weight within litter for survival in this line than the average birth weight, whereby this effect seems to be accounted for at individual piglet level. Individual birth weight is especially for the S-D line a preferable mean for improving survival at birth. The variation in parameters between lines indicates that the choice of the optimal selection for birth weight should be considered for each line individually to maximise overall genetic improvement in piglet survival and growth.

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A comparison of the performance of Holstein-Friesian and Norwegian Red dairy cattle on commercial dairy farms over five lactations

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Introduction Norway has adopted a multi-trait selection programme within its Norwegian Red (NR) dairy cow population for over thirty years, in contrast to breeding programmes with the Holstein-Friesian (HF) breed, which have, until recently, focused primarily on milk production. As a result, evidence from Norway suggests a dairy cow population with high levels of fertility and a relatively low incidence of mastitis. Consequently, there is considerable international interest in the NR breed as a means of overcoming some of the fertility, health and longevity problems that currently exist within the HF breed. To examine the potential of the NR breed, an experiment was established on 19 commercial dairy farms to compare the production, fertility and longevity of animals of the NR and HF breed across a range of production systems.

Material and methods Two hundred and twenty one NR dairy cattle were imported into Northern Ireland as maiden heifers (3 – 15 months old), and placed on 19 commercial dairy farms (11 or 12 animals/farm). An equal number of 'home bred' HF animals of similar ages were selected on each farm. More than 25 sires were represented within each breed. On each individual farm, animals of both breeds were subject to the same rearing and management regimes pre-calving, and to the same feeding regimes during lactation 1. During successive lactations, farmers managed individual cows within each breed as they believed to be appropriate. The 19 participating farms were selected to cover a range of production systems, and represented both Spring and Autumn calving herds. With the Holstein breed, 178, 135, 104, 72 and 47 cows completed lactations 1 – 5 respectively, while with the NR breed, the numbers were 193, 157, 127, 92 and 68 respectively. All farms participated in monthly milk recording schemes. Animal performance data within each lactation were analysed separately by ANOVA, with the effect of 'farm' included within the analysis. Within each lactation, 'days in milk' was used as a covariate when analysing full lactation milk yield and fat + protein yield data. The mean number of days in milk were 318, 303, 315, 321 and 306 for lactations 1 – 5 respectively. Performance of animals of each of the two breeds during lactations 1 – 5 are presented below.

Results While milk yields were significantly higher for the HF breed in lactation 1 ($P < 0.001$) and lactation 2 ($P < 0.05$), lactation milk yields did not differ between breeds in lactations 3, 4 and 5 ($P > 0.05$). Holstein-Friesian cows had a significantly ($P < 0.05$) higher lactation fat + protein yield than the NR cows in lactation 1, while there were no significant differences between breeds in fat + protein yield in any of lactations 2 – 5. NR cows has a significantly lower SCC than the HF cows in each of lactations 1 – 5 ($P < 0.05$ to $P < 0.001$). Concentrate intakes did not differ between breeds during any lactation ($P > 0.05$).

Table 1 Performance of Holstein-Friesian (HF) and Norwegian Red (NR) dairy cows on 19 commercial dairy farms

Lactation No	Full lactation performance	HF	NR	SED	Sig
Lactation 1	Milk yield (kg)	6264	5956	91.8	***
	Fat + protein yield (kg)	443	427	6.1	*
	SCC log10	1.93	1.77	0.039	***
	Concentrate intake (kg)	1451	1506	30.1	NS
Lactation 2	Milk yield (kg)	6789	6550	113.6	*
	Fat + protein yield (kg)	471	464	7.3	NS
	SCC log10	2.01	1.88	0.048	**
	Concentrate intake (kg)	1451	1422	40.2	NS
Lactation 3	Milk yield (kg)	7415	7257	137.3	NS
	Fat + protein yield (kg)	516	520	9.02	NS
	SCC log10	2.21	1.95	0.053	***
	Concentrate intake (kg)	1448	1505	45.4	NS
Lactation 4	Milk yield (kg)	7826	7506	188.0	NS
	Fat + protein yield (kg)	555	543	12.2	NS
	SCC log10	2.28	2.13	0.072	*
	Concentrate intake (kg)	1535	1481	51.5	NS
Lactation 5	Milk yield (kg)	7397	7466	225.0	NS
	Fat + protein yield (kg)	514	540	16.7	NS
	SCC log10	2.33	2.12	0.089	*
	Concentrate intake (kg)	1475	1584	66.0	NS

Conclusions Full lactation milk yields were higher with the HF breed in lactations 1 and 2, but not in lactations 3 – 5. NR cows produced a similar yield of fat + protein as the HF cows in each of lactations 2 – 5, while having a lower somatic cell count in all five lactations.

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A comparison of the reproductive performance of Holstein-Friesian and Norwegian Red dairy cattle on commercial dairy farms over five lactations

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Introduction Fertility traits have been included within breed selection programmes for the Norwegian Red (NR) dairy cow population in Norway for over thirty years. As a consequence, a 60 day non-return rate to first artificial insemination (AI) of 72.5% was recently calculated for NR cows in Norway (Garmo *et al.*, 2008). In view of this long term breeding programme, and current fertility performance within Norway, there is considerable interest in the NR breed as a means of overcoming the high levels of infertility that currently exist within the Holstein-Friesian (HF) breed. However, farming systems within Norway are very different from those within the UK, with herd sizes in particular being much smaller. Thus an experiment was established on 19 commercial dairy farms to compare the fertility performance of cows of the NR and HF breeds.

Material and methods Two hundred and twenty one Norwegian dairy cattle were imported into Northern Ireland as maiden heifers (3 – 15 months old), and placed on 19 commercial dairy farms (11 or 12 animals/farm). An equal number of 'home bred' Holstein-Friesian (HF) animals of similar ages were selected on each farm. More than 25 sires were represented within each breed. On each individual farm, animals of both breeds were subject to the same rearing and management regimes pre-calving, and to the same feeding regimes during lactation 1. During successive lactations, farmers managed individual cows as they believed to be appropriate. The 19 participating farms were selected to cover a range of production systems, and represented both Spring and Autumn calving herds. Throughout the study, animals were bred by both artificial insemination and by stock bulls, with breeding policy varying from farm to farm. A total of 46, 130, 96, 54 and 30 HF cows were inseminated by AI as heifers, and in lactations 1, 2, 3 and 4 respectively. The equivalent figures for the NR cows were 54, 154, 107, 71 and 45, respectively. Conception rates to 1st AI were calculated based on a subsequent calving. With the Holstein breed, 197, 157, 117, 88, 57 and 35 cows calved into lactations 1 – 6 respectively, while with the NR breed, the numbers were 208, 184, 144, 112, 78 and 59 respectively. Calving intervals were calculated for cows which calved into lactations 2 to 6. The proportion of cows culled as infertile between the start of the experiment and the start of lactation 6 was calculated. Binomial data (conception to 1st AI and proportion of cows culled) were analysed by binomial regression analysis, with breed differences compared using a t test. The effect of breed on calving interval was compared using REML analysis. In both cases, the analysis took account of farm effects.

Results Conception to 1st AI was significantly higher with the NR breed than with the HF breed in lactations 1 ($P<0.05$), 2 ($P<0.01$) and 3 ($P<0.001$), while not being affected by breed in maiden heifers and in lactation 4 ($P>0.05$). Calving interval was significantly lower with cows of the NR breed in lactation 3 ($P<0.05$), but not affected by breed in any other lactation ($P>0.05$). Of cows culled during the experiment (excluding cows culled for statutory reasons (Tuberculosis and Brucellosis) and due to farmers leaving the study), a significantly greater proportion of HF cows were culled due to infertility, compared to NR cows ($P<0.01$).

Table 1 Fertility performance of Holstein-Friesian (HF) and Norwegian Red (NR) dairy cows on 19 commercial dairy farms

	HF	NR	SEM	Sig
Conception to 1 st AI (proportion)				
Maiden heifers	0.58	0.66	0.065	NS
Lactation 1	0.41	0.55	0.040	*
Lactation 2	0.39	0.60	0.047	**
Lactation 3	0.35	0.65	0.058	***
Lactation 4	0.52	0.59	0.082	NS
Calving interval (days)				
Lactation 1	389	399	4.6	NS
Lactation 2	390	379	5.0	NS
Lactation 3	397	376	6.2	*
Lactation 4	387	384	7.1	NS
Lactation 5	399	386	8.3	NS
Proportion of cows culled during study ‡	0.76	0.71	0.025	NS
Of cows culled ‡, proportion culled due to infertility	0.42	0.25	0.039	**

‡Excludes cows culled for statutory reasons (Tuberculosis and Brucellosis) and due to farmers leaving the study

Conclusions Reproductive performance of NR dairy cows was significantly better than HF cows when managed under typical commercial farm conditions in Northern Ireland

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Including muscularity in Holstein-Friesian dairy breeding goals

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Introduction The role of the dairy herd as a provider of beef has proportionately reduced as the dairy animal has become more specialised over the years. High milk production per cow and a decreasing national herd has resulted in a reduction in calves born, and genetic changes have led to dairy bred bull calves being less suited to beef production. This has led to the market for purebred dairy bull calves being severely curtailed and, in some instances, the male purebred calf has had no value. The study develops tools to allow Holstein-Friesian (HF) dairy breeding goals to include muscling and carcass quality to help improve the value of dairy bred bull calves as beef animals.

Materials and methods The predicted transmitting abilities (PTAs) for carcass traits (Irish Cattle Breeding Federation) were correlated with those for production traits (milk, fat and protein) and fitness traits (somatic cell count, SCC; lifespan, LS; calving interval, CI; and non-return rate after 56 d, NR) produced in September 2007 (DairyCo breeding+). The carcass traits, estimated for HF sires in Ireland are carcass weight (CWT, kg), adjusted carcass conformation score (CCON), and adjusted carcass fat score (CFAT). The carcasses were classified at the abattoir using the EU beef classification scheme for conformation (EUROP) and fat class (1-5). The minimum reliability for inclusion in the correlation estimation for the carcass traits was 70-80% and for production and fitness traits 80-90%. The PTAs were used to estimate approximate genetic correlations by accounting for the reliability of the PTA in the estimation of the correlation (Hickman et al., 1969; Calo et al., 1973). The goal of the current national selection index for dairy cattle in the UK (£PLI) is profit and includes a range of production and fitness traits, including those listed above. Phenotypic and genetic parameters between the traits in £PLI and carcass conformation were collated from this and previous studies. Using selection index theory (Hazel, 1943) different indices were simulated that changed the goal of £PLI from profitability to a desired outcome for carcass traits. The carcass trait included in the goal was carcass conformation as this trait has the biggest impact on the final value of a carcass.

Results Table 1 shows that there is a moderate correlation between carcass traits and milk production with CCON and CFAT being negatively correlated with milk production traits suggesting that higher producing animals will have unfavourable carcass classification. There is a favourable correlation between milk production and CWT suggesting that higher milk production bulls will produce heavier animals at slaughter. There was no significant correlation between the carcass traits and LS and SCC. There was a significant low to moderate correlation between the fertility traits and CCON, suggesting that bulls producing more highly conformed carcasses will produce daughters that will have better fertility. However, this correlation may be driven through the negative relationship between milk production and CCON. Table 2 shows that selection on £PLI and an index that improves CCON leads to a favourable correlated response in carcass weight, ranging from 0.64 - 0.75 kg improvement per annum. As the desired rate of improvement in CCON increases the expected response in fitness traits (CI and mastitis) is favourable but the rate of improvement in milk production traits decreases.

Table 1 Approximate genetic correlations (standard errors in brackets) between carcass traits and production and fitness traits in HF animals.

	CWT (kg)	CCON (1-15)	CFAT (1-15)
Milk (305d, kg)	0.22 (0.064)	-0.40 (0.060)	-0.34 (0.062)
Fat (305d, kg)	0.23 (0.064)	-0.33 (0.062)	-0.33 (0.062)
Protein (305d, kg)	0.33 (0.062)	-0.26 (0.064)	-0.23 (0.064)
LS (lactation)	-0.07 (0.070)	0.05 (0.070)	0.06 (0.070)
SCC	-0.05 (0.067)	0.07 (0.067)	0.05 (0.067)
CI (days)	-0.04 (0.068)	-0.31 (0.064)	-0.25 (0.066)
NR (0/1)	-0.16 (0.067)	0.10 (0.068)	0.23 (0.066)

Table 2 Expected annual responses to selection in some production, fitness and carcass traits for indices improving carcass conformation

	Milk (kg)	Fat (kg)	Prot (kg)	LS (lacts)	Mastitis (cases)	CI (days)	CCON (1-15)	CWT (kg)
£PLI	40.79	3.40	2.72	0.060	0.0011	0.30	-0.033	0.64
Index with a change in CCON per annum of:								
0	35.11	3.27	2.65	0.054	0.0008	0.21	0.000	0.71
0.02	30.99	3.12	2.55	0.048	0.0007	0.16	0.020	0.74
0.04	26.18	2.91	2.40	0.042	0.0005	0.09	0.040	0.75
0.06	20.71	2.63	2.20	0.035	0.0003	0.03	0.060	0.75
0.08	14.43	2.29	1.95	0.027	0.0001	-0.04	0.080	0.73
0.1	7.26	1.86	1.62	0.017	-0.0002	-0.12	0.100	0.69

Conclusions Selection on an index that improves carcass conformation of HF beef by 0.1 unit will lead to a reduced rate of improvement in production traits and an improvement in fitness traits albeit with a reduction in the rate of improvement in lifespan. These results can be used to develop dairy indices that include “beef” traits in the overall goal.

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Genetic variation of rectal temperature and its relationship to milk production in Holstein dairy cows

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Introduction Milk production is significantly decreased by thermal stress. The survival and performance of an animal during heat stress periods depend on several weather factors, especially temperature and humidity. Researchers reported dramatic decreases in milk production as temperature rose above 30 degree of centigrade. Very high environmental temperature is common during the summer months in Iran. Rectal temperature is as indicator of heat tolerance and has been the most frequently used physiological variable for estimating heat tolerance in cattle. Some dairy cows are more heat tolerant and productive when subjected to heat stress. Identification and selection of heat stress resistant cattle offers the potential to increase milk yield in tropical environment. The aim of the present study was to estimate genetic parameters of heat tolerance and its relationship to milk production in Holstein Dairy Cows in Iran.

Materials and methods A total of 1140 Holstein Dairy Cows in 14 herds in Tehran province of Iran were used. Rectal temperature was measured during hours of maximum thermal stress (1300 to 1600 h) in summer. Rectal temperature was measured with digital thermometer inserted approximately 15 cm into the rectum. Ambient temperature and relative humidity were recorded when rectal temperature of cows was measured. Temperature humidity index (THI) was calculated from ambient temperature and relative humidity. The formula was used to calculate THI in the present study is $THI = 0.8 \times \text{ambient temperature} + \text{relative humidity} \times (\text{ambient temperature} - 14.3) + 46.3$. THI values were calculated so that each animal had one THI value. Rectal temperatures are not normally distributed and data were transformed by taking the log of rectal temperatures. General linear model (GLM) of log (rectal temperature) showed the main significant effects on rectal temperature are temperature humidity index and stage of lactation. Model was designed basis on information of GLM and the stage of lactation was considered as fixed effect and THI as covariate. Milk production of cows were available from Animal Breeding Centre of Iran and milk (305 2X ME) was used for genetic analysis of milk production and herd-year-season were considered as fixed effects. An animal model was used for two trait genetic analysing and variance-covariance component was estimated by restriction maximum likelihood (REML) method.

Results Average temperature humidity index (THI) value was 86.2 (± 2.7) and THI ranged from 80.6 to 92.2 in during of recording period. Average of rectal temperature was 38.82 (± 0.45) and it ranged from 38 to 40.46. Average of milk was 7849 (± 1122) and it ranged from 3398 to 11451. Heritability of rectal temperature and milk production were estimated 0.26 (± 0.08) and 0.18 (± 0.08) respectively. Phenotypic and genetic correlation between milk production and rectal temperature were estimated +0.07 and -0.37 respectively. Variance component of traits are given in Table 1.

Table 1 Variance component of milk production and rectal temperature

	Milk	Log(rectal temperature)
Additive genetic variance	222791.663	0.0000058
Heritability	0.18(± 0.08)	0.26(± 0.08)

Conclusion The upper critical THI for dairy cows is 72. These results showed that cows were under heat stress condition during the recording period and that the rectal temperature of cows can indicate resistance of cows to heat stress. Heritability of rectal temperature was medium and is fairly good for animal breeding programme. Genetic correlation between rectal temperature and milk production was medium and negative. This shows selection of animals for milk production in hot conditions could improve resistance of cows to heat stress. Heritability of rectal temperature and its negative genetic correlation with milk production show that is possible to include heat tolerance among selection objectives for cows in hot conditions. A regional progeny test in hot conditions to identify bulls that are productive and more heat tolerant could decrease problems of dairy herds in thermal stress conditions.

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Effect of rearing season on heritability of cocoon traits in silkworm

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Introduction Silkworms are agricultural insects producing silk fibres. Because of economic importance of sericulture, breeding programs are performed in order to improve bio-economic traits of lines (production and resistance traits) and also developing new lines containing interesting characteristics. Silkworms in Iran are reared twice a year, in spring and autumn. Spring is the natural rearing period of silkworms because of good environmental conditions. In autumn the quantity and quality of the cocoon produced by silkworm lines decreases due to increasing mortality and access to mulberry leaves with low nutritive quality. This decrease in performance will be lower in the lines which are genetically resistant to severe environmental conditions. Therefore, it is necessary to breed and develop new silkworm lines suitable for such a situation. It is obvious that silkworm lines having lower performance but higher resistance can be introduced for autumn season rearing. Appropriate breeding programs have to be defined and conducted for the autumn rearing lines. Thus, we have to estimate genetic parameters of autumn and spring lines separately when reared in each season. The present study was undertaken to identify heritability of important traits in the spring and autumn seasons for 6 silkworm lines.

Materials and methods Important traits of cocoon weight, cocoon shell weight and cocoon shell percentage were individually recorded during 13 generations (8 spring and 5 autumn rearing periods) for the Chinese origin lines of 110, 154 and Y, and Japanese origin lines of 107, 101433 and 153 and three-trait selection index programme was done. For each line, 80 male and 80 female cocoons with random mating formed the base population. In the following generations, 24 full sib families were reared for each line. After cocooning at the end of rearing, 30 male and 30 female cocoons were recorded in each family. Finally 80 male and 80 female cocoons in each generation were selected based on the considered selection method to produce next breeding generation. Genetic parameters of lines (variance components) were estimated using Derivative-Free Restricted Maximum Likelihood method using DFREML software (3.1 version) with 10^{-8} convergence. After collecting information in the successive generations with identified relationship among relatives. Heritability of traits was calculated as dividing additive genetic variance by phenotypic one (sum of additive genetic and error variances) using following mixed model for estimation of variances-covariance components: $y_{ijkl} = \mu_i + G_j + S_k + U_l + e_{ijkl}$ which y_{ijkl} = individual data for trait i , μ_i = average of trait i , G_j = effect of generation (year-season), S_k = effect of sex k (male and female), u_l = additive genetic value (random effect) for animal l , and e_{ijkl} = residual effects.

Results Heritability of traits are shown in Table 1. In spring and autumn seasons, the heritability of cocoon weight was higher in 154 (0.423) and 153 (0.606) lines, respectively while the lowest heritabilities were observed in Y (0.274) and 107 (0.201) lines, respectively. Heritability of cocoon weights in some lines (153 and Y) showed a sharp increase in autumn season (0.315 to 0.606 in 153, and 0.274 to 0.538 in Y lines). In four lines of 110, 101433, 153 and Y, cocoon weight heritability increased in autumn season compared with spring but this parameter was higher in spring in only 107 and 154 lines. The lines 101433 and 153 had the maximum heritability for cocoon shell weight in spring season (0.396) but in autumn, the largest belonged to Y line (0.469). 107 line showed the lowest heritability for cocoon shell weight in spring rearing season (0.259) but 107 and 101433 line demonstrated the lowest in autumn season (0.261). Except for 101433 line, the heritability of cocoon shell weight was higher in the autumn season. In spring and autumn, the maximum heritability for cocoon shell percentage was indicated in 101433 (0.248) and 154 (0.198) lines, respectively while the minimum was in 107 (0.174) and 101433 (0.133) lines, respectively. Except for 154 line, heritability of cocoon shell percentage, was higher in spring season than autumn. In 110, 101433, 153 and Y lines, heritability of cocoon shell weight was higher than cocoon weight in spring season but was lower than cocoon weight in autumn season. The reverse result was obtained in other two lines (107 and 154). Generally, cocoon shell percentage heritability was lower than other two traits.

Table 1 Heritability ($\pm$ standard error) of traits in spring and autumn rearing seasons for 6 silkworm lines

Line	Cocoon Weight		Cocoon Shell Weight		Cocoon Shell Percentage	
	Spring	Autumn	Spring	Autumn	Spring	Autumn
110	0.298 $\pm$ 0.021	0.321 $\pm$ 0.024	0.300 $\pm$ 0.020	0.304 $\pm$ 0.025	0.198 $\pm$ 0.017	0.167 $\pm$ 0.019
154	0.423 $\pm$ 0.033	0.329 $\pm$ 0.028	0.275 $\pm$ 0.019	0.334 $\pm$ 0.024	0.176 $\pm$ 0.019	0.198 $\pm$ 0.024
Y	0.274 $\pm$ 0.018	0.538 $\pm$ 0.030	0.357 $\pm$ 0.027	0.469 $\pm$ 0.026	0.185 $\pm$ 0.020	0.142 $\pm$ 0.018
107	0.310 $\pm$ 0.023	0.201 $\pm$ 0.011	0.259 $\pm$ 0.022	0.261 $\pm$ 0.021	0.174 $\pm$ 0.018	0.140 $\pm$ 0.016
101433	0.301 $\pm$ 0.030	0.318 $\pm$ 0.021	0.396 $\pm$ 0.031	0.261 $\pm$ 0.019	0.248 $\pm$ 0.024	0.103 $\pm$ 0.014
153	0.315 $\pm$ 0.034	0.606 $\pm$ 0.036	0.396 $\pm$ 0.033	0.461 $\pm$ 0.032	0.227 $\pm$ 0.023	0.133 $\pm$ 0.026

Conclusion From the obtained results, it was shown that as well as performance and genetic structure of silkworm lines, genetic parameters of cocoon characters are different in spring and autumn. Therefore, it is necessary to perform separate breeding strategies depending on the season the lines are reared. In most lines, heritability of cocoon weight and cocoon shell weight were higher in autumn while that of cocoon shell percentage was higher in spring.

Hindgut fermentation in dogs

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Introduction There is a large body of research investigating the nutritional needs of the dog at different life-stages and the use of different feed ingredients in commercial diets (Clapper *et al*, 2001; Schroeder & Smith, 2008). Despite this, though there has been relatively little published work looking at the role the large intestine plays in nutrient digestion in the dog. The dog's gastro-intestinal tract has been compared to the cat's in terms of relative length. However the cat is a true carnivore and has little need to ferment fibre from the diet, whereas the dog is a scavenger and will consume a much wider range of food, and therefore may need to ferment fibrous material from the diet. The dog may therefore require a more developed large intestine to harbour a microbial population to aid in the digestion of this fibrous material. This study was performed to determine differences in digestion of nutrients in this segment of the canine gastrointestinal tract.

Materials and methods Five purpose-bred adult female beagles with an average age of 3 ± 1.5 years and weight of 25.3 ± 4.6 kg were surgically prepared with ileal cannulas (according to the method of Walker *et al* 1994). Dogs were housed at the animal facility of the Edward R. Madigan Laboratory at the University of Illinois. The dogs were randomly assigned to two treatment groups and were fed two commercial AAFCO approved diets, one high and one lower in nutrient density, in a standard cross-over design with a 14-day feeding period for each diet. The dogs were fed twice daily and fresh water was available at all times. Each feeding period was split into a 10 day adaptation and a 4 day ileal and faecal collection period. Chromium oxide (Cr_2O_3) was used as an indigestible marker. From day 6 to 14 of each period, dogs were dosed with 0.5g of Cr_2O_3 via gelatine capsules at feeding (total daily intake of the marker was 1g).

Table 1 Nutrient composition of diet A and diet B

Diet	Dry Matter	% Dry matter basis		
		Crude Protein	Fat	Carbohydrate
Diet A	84.0	24.3	7.1	60.1
Diet B	93.9	29.0	12.5	51.5

Results The current study demonstrated significant differences ($P < 0.05$) in dry matter, crude protein and carbohydrate digestibility between samples collected from the terminal ileum and voided faeces from animals fed Diet A. There were also significant differences ($P < 0.05$) in the ileal dry matter, crude protein and carbohydrate digestibility between the two diets, but no differences in faecal digestibility.

Table 2 Apparent ileal and faecal digestibilities (mean $\pm$ SEM) of Diet A and B in dogs

		Dry Matter		Crude Protein		Fat		Carbohydrate	
Diet A	Ileal	58.37 ^{ax}	± 8.07	60.47 ^{ax}	± 9.62	92.93	± 2.70	78.66 ^{ax}	± 3.13
	Faecal	74.86 ^y	± 2.15	74.42 ^y	± 1.88	90.67	± 0.56	90.75 ^y	± 1.80
Diet B	Ileal	79.13 ^b	± 4.18	79.71 ^b	± 4.12	97.73	± 0.33	95.57 ^b	± 1.11
	Faecal	80.50	± 1.06	82.55	± 1.22	95.33	± 0.39	99.73	± 0.75

^a & ^b indicate significant difference between diets ($P < 0.05$) ^x & ^y indicate significant differences between ileal and faecal digestibility for each nutrient ($P < 0.05$)

Conclusions This study shows that there is significant fermentation of some nutrients in the hindgut of the dog. Interestingly, the effect is most apparent in the lower specification diet where protein and carbohydrate digestibility were significantly reduced in the small intestine. This in turn indicates that more nutrients were entering the hindgut and were available for fermentation. In contrast, both diets were relatively low in fat, and this nutrient was highly digestible so little or none reached the hindgut. This trial indicates that the hindgut of the dog is capable of utilising protein or carbohydrate entering this portion of the gastrointestinal tract.

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Immune enhancing potential of a canola oil-based supplement for the working dog

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Introduction The increasing use of functional foods as a non-invasive way to modulate and optimise human immune system function is now beginning to transfer into the production animal and companion animal areas as owners search for alternative ways of maintaining and improving animal health. To date, dietary supplementation trials, primarily carried out in humans and mice have demonstrated the ability of a wide variety of bioactive ingredients such as probiotics and specific ratios of omega-3 and omega-6 fatty acids, to modulate a range of immune functions. However, little data exists for other animal species such as the cat or dog and in particular the working dog. Working dogs are a vital component of the farming sector in New Zealand (NZ), however, many animals are subjected to inadequate nutrition that may compromise their short term performance and exercise recovery, and long term usefulness to the farmer (Jerram, 2005). In this study we assessed the potential benefits of dietary supplementation with a canola oil-based dietary supplement (canola is a rapeseed variant), formulated at Massey University, and manufactured in NZ, to enhance a number of immune parameters in the working dog.

Materials and methods This study using 12 adult, harrier hounds trained to run on a treadmill, was conducted at the Centre for Feline and Canine Nutrition, Massey University, Palmerston North, NZ. Dogs paired according to sex and age were randomly assigned to either the Test or Control treatment groups (n=6). A commercial dry biscuit diet (23.1% Protein, 18.7% Fat, 47.9% CHO, DM basis) was used as the basal diet and was fed according to maintenance (Burger, 1993), with either the canola oil-based supplement (palaMOUNTAINS MyBeau[®], VitaPower Ltd, Wanganui, NZ) added at a rate of 1.35g/kg body weight (Test group) or gelatinised corn starch added to make the diet isocaloric (Control group). The dogs were fed the diets for 56 days and had free access to water at all times. Body weight and body condition score (Purina Body Condition System; Laflamme, 1993), was monitored at weekly intervals throughout the experimental period. All the dogs were exposed to the same exercised regime. Blood samples were taken by jugular venepuncture on days 0, 14, 28 and 56 and collected into heparin tubes. These whole blood samples were used to assess immune cell responses, including lymphocyte proliferation to the T-cell mitogens phytohaemagglutinin (PHA) and concanavalin A (Con A) by measuring incorporation of ³H-thymidine, expression of the cell surface markers CD 4 (T-helper cell), CD 8 (cytotoxic T-cell), B cell, CD 14 (monocyte) and CD11b (neutrophil activation marker) by flow cytometry and phagocyte function also by flow cytometry. Analysis of variance (ANOVA, SAS, 1999) was used to determine significant differences.

Results The dogs remained healthy for the duration of the trial and maintained constant body weights and body condition scores. As shown in Table 1, dietary supplementation with palaMOUNTAINS MyBeau[®] resulted in a significant time dependant enhancement of lymphocyte proliferative responses to the T-cell mitogen PHA and a significant increase in phagocytic activity. A trend (P=0.066) of increased lymphocyte proliferative responses to Con A was observed after 8 weeks in the Test group. Control group animals showed no change in lymphocyte proliferative responses to either PHA or Con A during the experimental period. Dietary supplementation did not alter the level of expression of cell surface markers.

Table 1 Effect of dietary supplementation on immune parameters over time (* P<0.05, **P<0.01, ***P<0.001 cf time 0)

Time (weeks)	Lymphocyte Proliferation (PHA)				Phagocytic Activity			
	Control		Test		Control		Test	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
0	6.88	1.33	3.46	0.68	23.75	1.53	22.84	2.41
2	6.19	1.19	6.01	1.04	23.17	1.61	27.66	2.97
4	6.37	1.24	7.98	1.20*	22.57	1.79	30.13	2.80*
8	5.85	1.02	11.33	1.30***	22.33	1.80	33.76	2.36**

Conclusions Dietary supplementation with the canola oil-based supplement led to a time dependant enhancement of both lymphocyte proliferation and phagocytic activity. The lymphocyte proliferation results indicate that specific groups of T-cells were up-regulated by consumption of the supplement, priming them to proliferate in response to an appropriate antigenic challenge, such as a bacterial infection. The phagocyte function assay measures the ability of cells to ingest foreign particles such as bacteria. Since the enhanced phagocyte function occurred without any increase in the level of expression of CD 14 and CD 11b cells, the mechanism for the increase was not via increased numbers of monocytic cells or increased neutrophil activation. Taken together these results indicate that dogs given dietary supplementation with palaMOUNTAINS MyBeau[®] have significantly improved immune parameters and this may translate into having enhanced resistance and a greater ability to fight infection and disease.

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Investigation of indigestible markers in dogs

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Introduction Digestibility trials enable researchers to determine the amount of nutrients an animal absorbs and utilises from feed. There are a number of methods available, with the most common being the measurement of total feed intake and total collection of faeces. However, in situations where it is not practical to conduct a total collection trial, indigestible markers are used in conjunction with grab-sampling or sub-sampling techniques. To date, the most common marker used in dog studies has been chromium oxide (Cr₂O₃; Hill *et al.*, 1996), with little data available on the suitability of other markers such as titanium dioxide (TiO₂). A major problem in using Cr₂O₃ as an indigestible marker is poor repeatability and agreement between laboratories in the determination of Cr₂O₃. This has led to variable results due to incomplete and inconsistent recovery in excreta (Sales and Janssens, 2003). However work on other species comparing the use of TiO₂ and Cr₂O₃, has demonstrated that TiO₂ was a more accurate marker (Jagger *et al.*, 1992). This study was performed to determine the digestibility of two diets, a low (LN) and a high nutrient (HN) diet using TiO₂ or Cr₂O₃ as indigestible markers. The two different quality diets were used to investigate if digestibility affected marker recovery.

Materials and methods Twelve female adult Harrier hounds, aged between 2 and 7 years of age, with body weights ranging from 16.0 to 24.0kg (21.2 ± 2.1kg; mean ± SEM) were used in the study. The dogs were housed in pairs and the study was run as a 4 x 4 factorial design. At the start of each period, one dog per pen was allocated to one of four dietary treatments: LN + Cr₂O₃ (0.3% g/g as is): LN + TiO₂ (0.3% g/g as-is): HN + Cr₂O₃ (0.3% g/g as is): HN + TiO₂ (0.3% g/g as-is). Both the LN and HN diets had passed an AAFCO minimum feeding protocol for a complete and balanced adult maintenance diet (AAFCO, 2000). The LN diet contained 17.4% crude protein, 10.4% crude fat and 64.9% carbohydrate on a dry matter basis. The HN diet contained 26.7% crude protein, 16.2% crude fat and 49.7% carbohydrate on a dry matter basis. Each dog received all four diets over four 11 day evaluation periods in a latin square design. Each period comprised a 6 day adaptation period and a 5 day total faecal collection period, each period starting immediately after the previous one. Fresh water was available to the dogs at all times. SAS V8 statistical package was used in the data analysis of TiO₂ and Cr₂O₃ recoveries, which were subjected to analysis of variance with TiO₂ and Cr₂O₃ as fixed variables.

Results There were no significant differences (P>0.05) in the recoveries of the two indigestible markers from the diets.

Table 1 Indigestible marker recoveries (mean ± SEM) of 4 diets

	Diet					
	Cr ₂ O ₃	TiO ₂	LN + Cr ₂ O ₃	LN + TiO ₂	HN + Cr ₂ O ₃	HN + TiO ₂
Recovery (%)	67.6	77.6	76.6	74.5	58.6	80.6
±SEM (%)	15.1	16.0	15.4	12.1	7.9	19.2

The average dry matter digestibility of HN diet was 86.0% (± 1.7%) and LN was 79.4% (± 1.8%). These digestibilities are consistent with the quality of diets. There were no significant differences (P>0.05) between the digestibilities of the two diets.

Conclusions There were no significant differences in the recoveries of Cr₂O₃ or TiO₂ either within the same diet, or between the two nutrient levels. TiO₂ recoveries were consistent between both diets, whereas Cr₂O₃ recoveries were numerically higher in the low quality diet. This suggests that the higher protein and fat levels within the HN diet may have affected Cr₂O₃ recoveries; however the high degree of inter-animal variation precluded demonstration of significant differences. Hill *et al.*, (1996) showed that faecal recoveries of Cr₂O₃ were lower and more varied than ileal recoveries, even when collections are made over 4 days, suggesting some Cr₂O₃ may have been retained in the colon, and that Cr₂O₃ concentrations did not reach equilibrium levels during the faecal collection period. However, recoveries were not different between the diets in the study. Other studies have reported changes in faecal quality with different diets (Clapper *et al.*, 2001). Therefore it is possible that the markers may also have been retained in the gastro-intestinal system of the dogs in the present study or may have been excreted in the urine, both possible reasons why marker recovery did not reach 100%. In conclusion, it was demonstrated that TiO₂ may be able to be used as an indigestible marker in digestibility trials using dogs, with recoveries consistent across different quality diets, however more work is required to maximise marker recovery.

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The effects of herbage allowance and frequency of allocation to ewes in mid pregnancy on ewe and subsequent lamb performance

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Introduction Previous studies at this centre have shown that extended grazing ewes in mid (Keady *et al.*, 2007, Keady and Hanrahan 2009b) late (Keady *et al.*, 2007) or throughout (Keady *et al.* 2007, 2008, Keady and Hanrahan 2009a) pregnancy increased lamb birth and weaning weights relative to progeny from ewes which were housed unshorn. Stocking rate needs to be reduced dramatically to facilitate year round grazing (Keady *et al.* 2008) as herbage availability during early autumn (to prepare ewes for mating, finish remaining lambs and enable a sufficient proportion of the farm to be rested to build up sufficient herbage for extended grazing) is the major limitation (Keady and Hanrahan, 2007). Keady and Hanrahan (2009b) showed that increasing herbage allowance to ewes in mid pregnancy decreased herbage utilisation but increased forage intake, ewe condition and subsequent lamb birth and weaning weights. The aim of the current study was to evaluate the effects of herbage allowance and frequency of allocation to ewes in mid pregnancy on ewe and subsequent lamb performance. Furthermore the effects of extended grazing management on subsequent herbage yield and ground cover were also evaluated.

Methods and materials Crossbred ewes [(52 Belclare x Scottish Blackface and 20 Chamoise x Scottish Blackface) (52 1.5 year olds, 20 4.5 year olds) (initial liveweight 68.4 kg, initial condition score 3.7)] due to lamb in mid March were allocated at random, balanced with respect to breed and age to four treatments in a randomised study. The four treatments were extended grazed pasture offered at dry matter (DM) allowances of 1.0 or 1.8 kg per ewe daily and allocated either daily or twice weekly from 28 November to 30 January. From 31 January to lambing the ewes were housed in pens of 5 and offered grass silage *ad-libitum* supplemented with a total of 19 kg concentrate per ewe. A blood sample was taken from the jugular vein of each animal on four consecutive days during weeks 3 and 9 of the extended grazing period to correspond with 0, 24, 48 and 72 h after herbage allocation for animals moved twice weekly and after 24 h for the animals moved weekly. Ewes and their lambs were turned out to pasture within 3 days of lambing. Ewes rearing singles and twins received no concentrate post lambing. Ewes rearing triplets received 1 kg concentrate/head daily for 3 weeks and 0.5 kg concentrate/head daily for weeks 4 and 5 post lambing. Concentrate was offered to lambs reared as triplets, to a maximum of 300 g/lamb daily until weaning. All lambs were weaned at 14 weeks. The data were analysed using Proc GLM and Proc MIXED of SAS as appropriate

Results There were no herbage allowance by frequency of allocation interactions in the current study. The DM, crude protein, acid detergent fibre and ash concentrations of the extended grazed herbage were 123 g/kg, 200 g/kg DM, 263g/kg DM and 117 g/kg DM respectively. The effects of herbage allowance and frequency of allocation on ewe and subsequent lamb performance are presented in Table 1. Increasing herbage allowance increased ewe condition score at the end of extended grazing and at weaning, and plasma total protein concentration, decreased plasma NEFA concentration, and tended to decrease the number of lambs born dead. Herbage allowance did not alter lamb birth or weaning weights. Twice weekly herbage allocation increased lamb birth weight and blood NEFA. Neither herbage allowance nor frequency of allocation altered ($P>0.05$) silage intake during late pregnancy. The effects of extended grazing management on subsequent herbage yield are presented in Table 2. Increasing herbage allowance at grazing increased subsequent herbage yield and decreased the proportion of bare ground. Frequency of herbage allocation did not alter ($P>0.05$) subsequent herbage yield or the proportion of bare ground.

Table 1 The effects of herbage allowance and frequency of allocation on ewe and subsequent lamb performance.

Frequency of allocation (F)	Herbage allowance (HA) (kg DM/d)					Significance		
	1.0		1.8		s.e.	HA	F	HA x F
	Daily	2 x weekly	Daily	2 x weekly				
Ewe condition score- end of EG ¹	2.97	3.00	3.24	3.15	0.094	**	NS	NS
- weaning	2.95	2.99	3.27	3.34	0.205	*	NS	NS
Litter size	1.90	1.75	1.84	1.98	0.170	NS	NS	NS
No born dead	0.20	0.13	0.00	0.04	0.105	P=0.06	NS	NS
Lamb - Birth weight (kg)	4.72	4.84	4.66	5.31	0.197	NS	*	NS
- Weaning weight (kg)	32.4	32.6	33.1	31.3	1.02	NS	NS	NS
Plasm - Total protein (g/l)	69.2	71.0	73.3	72.7	0.72	***	NS	NS
a								
- NEFA (m mol/l)	0.84	1.12	0.67	0.87	0.045	***	***	NS

¹EG = extended grazing

Table 2 The effects of extended grazing management on subsequent herbage yield

	Herbage allowance HA (kg/d)		Frequency of allocation (F)		Significance		
	1.0	1.8	Daily	2 weekly	HA	F	HAxF
DM yield (t/HA)	1.80	2.40	2.07	2.11	*	NS	NS
Bare ground (%)	14.1	5.9	9.9	10.1	*	NS	NS

Conclusions To maximise stock carrying capacity and reduce labour requirement and herbage allowance of 1 kg DM offered twice weekly is adequate for ewes in mid pregnancy.

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Effects of replacing grass silage with maize silage or concentrates on lamb output from housed pregnant ewes

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Introduction Rations for pregnant ewes in the UK are often based on grass silage. However, due to a number of factors, the cost of producing high quality grass silage has increased significantly in recent years so lower cost alternatives need to be investigated. Production of forage maize has the potential to reduce forage costs on mixed beef/sheep farms, with high dry-matter yields (comparable to that of a 3-cut silage system) of high quality maize silage possible from a single harvesting operation (Easson and Fearnough, 2000). However there is limited information on the effects of feeding maize silage to pregnant ewes. Alternatively, grass silage could be eliminated from rations by feeding high grain diets. The aims of this study were to evaluate the effects of replacing grass silage with either maize silage or concentrates on the performance of housed pregnant ewes and their lambs.

Materials and methods Seven weeks prior to lambing, 104 twin-bearing ewes (80 ± 10.2 kg live weight, condition score 3.8 ± 0.21) were housed, shorn and allocated to one of four treatments, balanced for live weight, body condition and crossing sire breed. For the final 6 weeks of pregnancy, ewes were offered one of four diets: precision chop grass silage + 0.55 kg/d concentrate (GS, Treatment 1); maize silage + 0.55 kg/d concentrate (MS, Treatment 2); a mixture (1:1 on a dry-matter basis) of grass silage and maize silage + 0.55 kg/d (GS/MS, Treatment 3); or 1.55 kg/d concentrates + 50 g/d chopped barley straw (C, Treatment 4). All silages were offered on an *ad libitum* basis. The grass silage was predicted by Near Infrared Reflectance Spectroscopy to supply 278 g DM/kg, 670 g/kg digestible organic matter/kg DM, 10.7 MJ ME/kg DM and 121 g crude-protein/kg DM. Maize silage was predicted to supply 337 g DM/kg, 11.0 MJ ME/kg DM, 84 g CP/kg DM and 243 g starch/kg DM. Concentrates were formulated to supply 209, 293, 242 and 158 g CP/kg DM for Treatments 1-4 respectively, with the aim of achieving a total intake of 130 g metabolisable protein/ewe/day. Half of the ewes were housed individually and intakes were recorded daily for each animal. The remainder were housed in groups of 4-5, with intakes recorded daily for the entire group. Ewe live weight and condition score were measured 6, 4 and 2 weeks pre-lambing and within 24 h of lambing. Lambs were weighed at birth, 6 weeks of age and weaning. Daily live weight gain was determined by linear regression. Lambing difficulty was scored on a four-point scale where 1 = no assistance and 4 = manual delivery with difficulties. Data were analysed using Analysis of Variance (ANOVA) with diet as a fixed effect and covariates included for crossing sire breed, lamb sex and age at weaning, where appropriate.

Results Ewes offered maize silage as 0.5 or 1.0 total forage had higher intakes of silage DM ($P < 0.01$) and total DM ($P < 0.001$) during the final 6 weeks of pregnancy compared with ewes offered grass silage as the sole forage. Ewes offered the complete concentrate diet had the lowest DM intake. However there was no evidence of any dietary effects on the nutritional status of ewes, in terms of changes in live weight or condition score. Diet in late pregnancy had no significant effects on lamb output at birth or at weaning. However there was a higher incidence of lambing difficulties with MS compared with GS or C ewes, as shown by their higher mean lambing difficulty score. There were no residual effects of late pregnancy diet on lamb performance.

Table 1 Effects of replacing grass silage with maize silage or concentrates on ewe and lamb performance

	Grass Silage	Grass Silage + Maize Silage	Maize Silage	Concentrates + Straw	s.e.d	Sig
Forage dry-matter intake (kg/d)	1.03 ^a	1.18 ^b	1.15 ^b	-	0.046	**
Total dry-matter intake (kg/d)	1.50 ^b	1.64 ^c	1.62 ^c	1.21 ^a	0.041	***
Pre-lambing live weight change (kg)	-6.1	-4.2	-5.4	-7.4	1.36	NS
Pre-lambing condition score change	-0.46	-0.52	-0.50	-0.44	0.155	NS
Mean lamb birth wt (kg)	5.38	5.40	5.22	5.41	0.223	NS
Lambing difficulty score	1.46 ^a	1.53 ^{ab}	1.93 ^b	1.10 ^a	0.229	**
Lambs weaned/ewe	1.62	1.84	1.60	1.77	0.149	NS
Total weaning wt (kg)	57.8	64.1	55.1	60.4	4.87	NS
Daily live weight gain (g/d)	244	257	245	250	8.0	NS

Conclusion The results of this study demonstrate that maize silage can replace grass silage in pregnant ewe diets with no adverse effects on lamb output. However the higher intake characteristic of maize silage, without a concomitant increase in lamb output, suggests that feed conversion efficiency is lower than for grass silage. Also the higher incidence of lambing difficulties in ewes offered maize silage is a major concern and requires further investigation. Zero silage systems based on high concentrate inputs can also replace grass silage-based diets without affecting lamb output, although the economics of this system is dependent on concentrate feed costs.

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Effects of altering the plane of nutrition during the rearing phase and pregnancy of 2-tooth ewes, of two genotypes, on ewe and subsequent lamb performance

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Introduction Ewe replacements are a major cost in sheep production. This cost is influenced by replacement rate and rearing expense which is a function of plane of nutrition offered during the first winter, subsequent grazing season prior to joining at 18 months of age and during pregnancy prior to lambing as 2-tooth ewes. There is a paucity of data on the impact of plane of nutrition between 6 months of age and first lambing as 2-tooth ewes on body size and subsequent productivity. The weight of lamb carcass produced per ewe, which is influenced by litter size, lamb survival and growth rate, is the main factor affecting output per ewe. Plane of nutrition at different stages during the rearing phase, genetic potential for prolificacy and their potential interactions may be important. The aim of the current study was to evaluate effects of plane of nutrition during the first winter, subsequent grazing season and during first pregnancy, and potential interactions, on ewe body size and productivity and subsequent lamb performance using two breed types with contrasting prolificacy potential.

Materials and methods Ewe lambs [60 Chamoise x S. Blackface, 34 Belclare x S. Blackface; mean initial live weight 34.8 and 39.9 kg, respectively] were allocated to two herbage dry matter (DM) allowances daily [0.75 kg (L) and 1.75 kg (H) per head] on winter grazing (winter-1), from 25 Nov to 29 Mar. From 29 Mar to 30 Aug half of the ewe lambs from each winter treatment were allocated one of two planes of summer nutrition by set stocking to maintain sward heights of 4 cm (L) or 6 cm (H) (summer). From 30 August to early December all ewes were grazed as one flock. At housing half of the ewes on each of the four treatments were allocated either high (H) or low (L) feed value grass silage (winter-2). Thus, there were eight treatment groups [(2 winter-1 treatments (HL) x 2 summer grazing treatments (HL) x 2 winter-2 treatments (HL)]. During the last 6 weeks of pregnancy the ewes were offered 20 kg concentrate per head. Ewes and their lambs were turned out to pasture within 3 days of lambing. Ewes rearing singles or twins were offered 0.5 kg concentrate/d for 5 weeks post lambing while their lambs were offered up to 300 g/d until weaning. Lambs received no concentrate post weaning. Pre-experimental measurements were used as covariates in the analyses of weight and linear measurements. The data were analysed using Proc GLM and Proc MIXED of SAS as appropriate.

Results The effects of plane of nutrition at different stages during the rearing phase until lambing as 2-tooth ewes on ewe and subsequent lamb performance are presented in Table 1. Increasing winter-1 plane of nutrition increased ewe weight and condition at lambing, lamb birth weight and tended to increase ($P=0.08$) lamb weight at weaning. Increasing winter-2 plane of nutrition increased ewe condition and live weight at lambing and lamb daily gain from birth to weaning. Plane of nutrition during the summer did not affect ($P>0.05$) ewe performance or that of their lambs. The effects of ewe genotype on ewe and subsequent lamb performance are presented in Table 2. Relative to the Chamoise-X, the Belclare-X were heavier at lambing, produced larger litters and reared lambs which were heavier at birth and weaning.

Table 1 Effect of plane of nutrition during the rearing phase on ewe and subsequent lamb performance

Table 1 Effect of plane of nutrition during the rearing phase on ewe and subsequent lamb performance														
		Winter-1 plane of nutrition (W1)								s.e.	Significance			
		Low				High								
		Low		High		Low		High						
Summer (S)	Winter-2 (W2)	Low	High	Low	High	Low	High	Low	High		W1	S	W2	W1xS
	Post lambing -condition	2.56	3.48	2.59	3.38	2.87	3.59	2.89	4.14	0.142	***	NS	***	NS
	- weight (kg)	50.9	54.5	47.2	55.0	50.4	57.3	56.9	64.5	2.09	***	P=0.08	***	**
	Litter size	1.89	1.50	1.60	1.52	1.50	1.61	1.57	1.59	0.152	NS	NS	NS	NS
	Lambweight (kg) - birth	4.6	4.6	4.2	4.9	4.7	5.0	5.1	4.9	0.19	**	NS	NS	NS
	-weaning	27.7	29.3	27.8	29.5	30.4	31.2	28.6	30.6	1.19	*	NS	NS	NS
	Lamb gain (g/d)	241	260	239	253	256	269	240	264	9.8	NS	NS	*	NS

¹There were no ($P>0.05$) winter 1 x winter 2, summer 2 x winter 2 or winter 1 x summer 2 x winter 2 interactions

Table 2 Effect of ewe genotype on ewe and lamb performance

	Ewe genotype		s.e.	Sig.
	Chamoise-X	Belclare-X		
Post lambing -condition	3.03	2.72	0.075	***
- weight (kg)	51.7	57.5	1.06	***
Litter size	1.34	1.85	0.078	***
Lamb weight (kg) -birth	4.55	4.94	0.101	**
- weaning	28.1	30.7	0.62	**
Lamb gain (g/d)	239	266	5.5	**

Conclusions Whilst altering the plane of nutrition during different stages of the rearing phase increased ewe liveweight by up to 36%, there was no effect on litter size or number reared. Plane of nutrition during the first winter had the greatest effect on subsequent lamb birth and weaning weights. The Belclare-X increased the weight of lambs weaned per ewe by 67% due to a combination of higher litter size (0.51) and higher progeny growth rate (+30 g/d).

Performance of crossbred Welsh Mountain ewes in the hill environment

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Introduction The Welsh Mountain sheep is a numerically important breed in the UK, but its lambs are characterised by low slaughter weights and poor carcass conformation. The reduction in stocking rates that is often associated with environmental conservation schemes, offers the potential for the use of larger ewes. Crossbreeding allows more rapid genetic change to be undertaken than would be possible from selection within breeds, and affords the potential to utilise heterosis and improve the quality of subsequent crosses with the Bluefaced Leicester. This work compared the survival and productivity of purebred Welsh Mountain ewes with a range of crossbred ewe types maintained in a hill environment.

Materials and methods Over a three year period, 600 Welsh Mountain ewes per year were mated to either Cheviot, Poll Dorset, Lleyn or Texel sires (8 high index sires per breed overall) using laparoscopic AI (McLean et al, 2006). Up to fifty ewe lambs per year were retained from each breed and a similar number of pure bred Welsh Mountain lambs were also retained each year from the farm flock. These pure Welsh Mountain and crossbred ewes were mated by Suffolk rams and ewe performance was recorded over 3 parities. Ewes were weighed and condition scored on a scale of 1 (thin) to 5 (fat) at the start of the mating period. All ewes in their first parity (two years old) were housed for the final 6 weeks of pregnancy and fed grass silage and concentrates according to predicted litter size. In subsequent parities only twin and triplet bearing ewes were housed, and single bearing ewes were maintained on pastures and fed grass silage supplemented with feed blocks until lambing. After lambing, single bearing ewes were grazed on semi-natural hill pastures at 1.5 ewes per ha, whilst twin rearing ewes were retained on improved perennial ryegrass based swards at up to 10 ewes per ha until lambs were weaned at 18 weeks of age. The data were analysed using the REML procedure of Genstat. For ewe performance traits, the fixed effects of breed, year, ewe age and their interactions were evaluated. Lamb performance traits were adjusted for the fixed effects of breed of dam, year, sex, litter size, ewe age and significant interactions, with age of lamb at the 8 week weighing and/or weaning fitted as covariates where relevant. Dam was fitted as a random effect.

Results There were no significant differences ($P>0.05$) between ewe types in pregnancy rates and survival to third lambing. Cheviot, Dorset and Texel crossbred ewes were approximately 8 kg (proportionately 0.2) heavier than the Welsh Mountain ($P<0.001$) and Lleyn cross ewes were intermediate in weight. Relative to the number of ewes that entered the flock, total litter weight weaned over three lamb crops was between 18.7 - 31.3 kg higher in crossbred ewe types than in the Welsh Mountain ($P<0.001$), although the relative contribution to this of litter size and individual lamb weaning weights varied between crossbreeds. The progeny of crossbred ewes were heavier at birth and weaning than lambs from Welsh Mountain ewes. However, although crossbred ewes supported higher growth rates in early lactation, there were no significant differences in ADG from 8 weeks to weaning ($P>0.05$).

Table 1 Performance of purebred Welsh Mountain and crossbred ewes and their Suffolk cross lambs

	Cheviot x	Dorset x	Lleyn x	Texel x	Welsh	s.e.d.	Significance
Number of ewes	135	136	145	130	126		
Ewe survival to 3 rd lambing	0.72	0.64	0.74	0.62	0.61	0.056	n.s.
Ewe live weight at 3 rd mating	50.3	50.9	46.9 ^b	51.2	42.5 ^a	0.86	$P<0.001$
Litter weight weaned [†] (kg)	97.1 ^a	103.3 ^{bc}	109.7 ^c	100.5 ^b	78.4 ^a	4.59	$P<0.001$
Pregnancy rate	0.85	0.86	0.86	0.85	0.81	0.030	n.s.
No lambs born/ewe lambing	1.49 ^{bc}	1.57 ^{cd}	1.63 ^d	1.43 ^b	1.33 ^a	0.049	$P<0.001$
No lambs reared/ewe lambing	1.42 ^{bc}	1.50 ^{cd}	1.52 ^d	1.37 ^{ab}	1.29 ^a	0.047	$P<0.001$
Lamb birth weight (kg)	3.8 ^{bc}	3.9 ^{cd}	3.7 ^b	4.0 ^d	3.3 ^a	0.07	$P<0.001$
Lamb weaning weight (kg)	29.4 ^b	30.4 ^c	29.2 ^b	30.7 ^c	27.0 ^a	0.36	$P<0.001$
ADG birth – 8 weeks (kg/day)	0.26 ^b	0.27 ^c	0.26 ^b	0.26 ^{bc}	0.24 ^a	0.006	$P<0.001$
ADG 8 weeks – weaning (kg/day)	0.14	0.15	0.15	0.15	0.13	0.005	n.s.

^{a, b, c} Means within a row with different superscripts were significantly different ($P<0.05$)

[†] Total litter weight produced over three parities per ewe that entered the flock

Conclusions These results are similar to those of Speijers *et al* (2007) for crossbred Scottish Blackface sheep and demonstrate that, where stocking rates are adequate, larger, more productive crossbred ewes can be maintained in the hill environment without deleterious consequences for survival or pregnancy rate.

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Lifetime performance of crossbred ewes in the hill sheep sector

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Introduction Hill sheep flocks in the UK are dominated by purebred ewe genotypes, with the Scottish Blackface being the most common. However a long term decline in economic returns, combined with recent changes to the Common Agricultural Policy, has lead many hill sheep producers to consider keeping crossbred ewes to exploit the benefits of hybrid vigour for lamb survival and to introduce beneficial traits for prolificacy, ease of lambing and carcass quality. In 2001, a major on-farm research programme was initiated to evaluate the performance of a range of crossbred ewe genotypes for the Northern Ireland hill sheep sector. Provisional data has already identified that retaining Lley 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). However, ewe longevity is a major issue for hill flocks, where the annual replacement rate often exceeds 20%. Therefore it is inappropriate to evaluate crossbred ewe genotypes based on average annual performance alone, and the aims of this study were to investigate the lifetime performance of a range of crossbred genotypes under hill conditions.

Materials and methods This study was carried out on six hill farms, representative of the main hill farming regions of Northern Ireland. From November 2001-2003, 200 purebred Blackface ewes per farm were allocated to one of five mating groups, balanced for ewe live weight, body condition score and age. Each year, ewes were mated with Blackface (B), Cheviot (CH), Lley (LL), Swaledale (SW) and Texel (T) rams. In 2001, single sire mating groups were used on each farm while in 2002 and 2003, a team of rams was used across all farms using artificial insemination. A total of 15 rams from each of the five sire breeds were used to produce the crossbred progeny. Where possible, rams were selected on the basis of their genetic indices to represent the top 10% of recorded animals for the breed. The female progeny from these crosses were retained for breeding, which commenced at approx. 18 months old. Over a 4-year period (November 2003-2006), the crossbred ewes were mated in single sire mating groups to a range of sire breeds, which were balanced across all genotypes. Ewes were weighed prior to mating, 6 weeks pre-lambing, 6 weeks post-lambing and at weaning. Pregnancy was diagnosed each year by ultrasound scanning at day 80-100 of gestation. Ewes that were barren as hoggets were retained until the following year; however barren ewes >2 years old were culled after scanning. Ewes with functional abnormalities at weaning were also culled. Lambs were weighed at birth, 6 weeks of age and at weaning. Total lifetime output per ewe was calculated for each breed as the product of the proportion of productive ewes and the mean productivity per ewe lambled. The proportions of productive ewes at each lambing were estimated from a binomial regression analysis using a model with fixed effects for farm and breed. Mean productivity per ewe lambled were estimated using Repeated Measures Analysis of Variance. The model included fixed effects for farm, breed, parity and the breed x parity and breed x farm interactions.

Results Longevity of all crossbred ewe genotypes, in terms of the proportion of ewes remaining at the 4th parity, were similar to that of purebred Blackface ewes. However, over a 4 year period, the total number of lambs born ($P<0.05$) and the total weight of lambs born ($P<0.05$) per ewe were significantly higher in SWXB, LLXB and TXB ewes than BXB and CHXB ewes. These genotype effects remained at weaning, with SWXB, LLXB and TXB ewes successfully rearing a greater total number ($P<0.05$) and total weight ($P<0.05$) of lambs. These genotypes were also superior in terms of output efficiency, achieving a higher total lamb output/kg metabolic live weight ($W^{0.75}$) than BXB and CHXB ewes due to their greater weaned lamb output.

Table 1 Effects of hill ewe genotype on ewe productivity over a 4-year period

Ewe genotype	BXB	SWXB	CHXB	LLXB	TXB	s.e.d	Sig
Prop. ewes surviving to 4 th parity	0.59	0.61	0.51	0.57	0.62	0.076	NS
Total no. lambs born/ewe	4.63 ^a	5.32 ^b	4.64 ^a	5.23 ^b	5.01 ^{ab}	0.199	*
Total lamb birth weight (kg/ewe)	17.6 ^a	20.4 ^b	18.8 ^a	20.3 ^b	20.7 ^b	0.804	*
Total no. lambs weaned/ewe	3.93 ^a	4.61 ^b	3.96 ^a	4.48 ^b	4.34 ^b	0.191	*
Total lamb weaning weight (kg/ewe)	116 ^a	136 ^b	123 ^a	138 ^b	136 ^b	5.8	*
Total weaning weight/kg $W^{0.75}$	5.60 ^a	6.62 ^b	5.55 ^a	6.59 ^b	6.19 ^b	0.281	*

BXB, Scottish Blackface; SWXB Swaledale X Blackface; CHXB, Cheviot X Blackface; LLXB, Lley X Blackface; TXB, Texel X Blackface; $W^{0.75}$, metabolic live weight (W = live weight at mating). Means sharing the same superscript are not statistically significant ($P>0.05$)

Conclusion The results of this study demonstrate that adopting a crossbreeding strategy on hill flocks to produce crossbred replacement ewes is unlikely to have any adverse effects for ewe longevity and flock replacement rate. However lifetime lamb output of hill ewes could be increased by up to 20% by crossing Blackface ewes with Lley, Swaledale or Texel sires to produce flock replacements, rather than maintaining a purebred status. Therefore crossbreeding has a key role to play to help ensure that the UK hill sheep sector remains economically viable in the future.

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Effects of mild early- and mid-pregnancy under-nutrition on foetal and placental development in Scottish Blackface and Suffolk sheep

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Introduction Nutrient intake during pregnancy affects foetal development and placental function in a range of species, often with long-term effects on offspring viability. Maternal nutrient supply is believed to affect the ability of the placenta to deliver nutrients to the foetus (Fowden et al., 2006). In ruminant species, the majority of placental nutrient transport occurs in specialised structures called placentomes, which are categorised into 4 types (A-D) based on their morphological appearance. In type A placentomes, maternal tissue surrounds foetal tissues, whereas type D placentomes are typically everted and have a higher ratio of foetal:maternal tissue. It has been suggested that the distribution of placentome types may reflect the ability of the placenta to deliver nutrients (Vonnahme et al., 2006). This study tested the hypothesis that levels of under-nutrition typically encountered by hill ewes during pregnancy would alter the distribution of placentome types and that the relationship between ewe nutrition and placentome type would differ between breeds selected for lean tissue growth (such as the Suffolk) compared to less selected breeds (such as the Scottish Blackface).

Materials and methods Oestrous cycles of 18 month-old Suffolk (S) and Scottish Blackface (B) ewes were synchronised and ewes were inseminated with semen from a single sire of each breed. All ewes received concentrate and straw-based rations from days 1 to 90 of gestation inclusive. During this time, half the ewes received a restricted diet calculated to supply 0.8 x maintenance energy requirements (R), while the remaining ewes received control (C) rations supplying 1.0 x maintenance energy requirements. From Day 91, all ewes received hay *ad libitum* and concentrates formulated to meet requirements for the stage of pregnancy. On day 125 of gestation 6 single-bearing ewes of each breed x nutrition combination were euthanized. Weights of the gravid uterus, placenta and foetus were recorded together with foetal crown-rump length. All placentomes were dissected and classified as A, B, C or D as described by Vatnick et al. (1991). Effects of nutrition and breed were analysed in a 2x2 ANOVA using the proc GLM procedure in SAS. Non-normally distributed data were square root or log transformed prior to analysis.

Results In all ewes studied the majority of placentomes (>60%) were of type A. There was no effect of nutritional treatment on the total number of placentomes or on the distribution of placentome types in either breed. The breed of ewe affected both the number of placentomes and individual placentome weight (Table 1).

Table 1 Effects of breed on placentome number and weight on day 125. Data show means $\pm$ s.e.m.

	Scottish Blackface		Suffolk		P-value
	Control	Restricted	Control	Restricted	Breed
Number of placentomes	106 $\pm$ 4.58	106 $\pm$ 7.78	95 $\pm$ 4.26	95 $\pm$ 2.14	0.04
Total placentome weight (g)	464.8 $\pm$ 18.23	471.0 $\pm$ 19.26	599.6 $\pm$ 59.90	587.2 $\pm$ 24.5	<0.01
Mean placentome weight (g)	4.4 $\pm$ 0.23	4.5 $\pm$ 0.33	6.4 $\pm$ 0.72	6.2 $\pm$ 0.34	<0.01
<i>Mean weight per placentome type (g)</i>					
Type A	3.9 $\pm$ 0.35	4.2 $\pm$ 0.40	4.7 $\pm$ 0.77	4.9 $\pm$ 0.28	0.13
Type B*	2.4 $\pm$ 0.09	2.3 $\pm$ 0.12	2.6 $\pm$ 0.18	2.8 $\pm$ 0.18	0.02
Type C**	1.8 $\pm$ 0.19	1.8 $\pm$ 0.14	2.3 $\pm$ 0.09	2.3 $\pm$ 0.22	0.01
Type D	4.4 $\pm$ 2.03	3.9 $\pm$ 1.73	8.9 $\pm$ 0.95	6.7 $\pm$ 0.80	0.02

* Square root transformed ** Log transformed

In both breeds the crown-rump lengths of foetuses carried by C ewes tended ($P < 0.1$) to be shorter than those carried by R ewes (B: 39.0 $\pm$ 0.62cm versus 39.87 $\pm$ 0.59cm, S: 43.12 $\pm$ 0.91cm versus 44.62 $\pm$ 0.61cm). There were no effects of treatment or breed on foetal weight as a proportion of maternal body weight, or on the ratio between foetal and placental weight. There were no significant breed x treatment interactions.

Conclusions These data suggest that in both breeds studied, the ability of the placenta to transport nutrients was not compromised by modest reductions in feed intake during the first 90 days of pregnancy. These results contrast with those of Vonnahme et al. (2006) who reported an increase in the proportion of type B, C and D placentomes on Day 78 in ewes fed 50% of NRC recommendations from day 28 – 78 of pregnancy. The ability of placental function to cope with reduced maternal feed intake may reflect the level and/or duration of under nutrition and maternal body reserves.

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Effects of early- and mid-pregnancy under-nutrition on maternal and foetal plasma free amino acid concentrations in Scottish Blackface and Suffolk sheep

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Introduction Nutrient intake during pregnancy affects foetal development and placental function in a range of species, often with long-term effects on offspring viability. Maternal supply and placental transport of amino acids are key to delivering amino acids for foetal metabolism. Maternal under-nutrition has been shown to reduce both maternal and foetal amino acid concentrations even after a period of re-feeding (Kwon et al. 2004). Intriguingly whether sheep have been adapted to a harsh environment or not appears to influence the extent of any reduction (Jobgen et al. 2008). The present study therefore tested whether plasma amino acid concentrations differed between Scottish Blackface (B) sheep adapted to a hill environment and a lowland breed (Suffolk, S) selected for lean tissue growth when challenged by a period of under-nutrition.

Materials and methods Oestrous cycles of S and B ewes (~18 month old) were synchronised and ewes were inseminated with semen from a single sire of each breed. All ewes received concentrate and straw-based rations from day 1 to day 90 of gestation inclusive. During this time, half the ewes received a restricted diet calculated to supply 0.8 x maintenance energy requirements (R), while the remaining ewes received control (C) rations supplying 1.0 x maintenance energy requirements. From day 91, all ewes received hay *ad libitum* and concentrates formulated to meet requirements for the stage of pregnancy. On day 125 of gestation, six single-bearing ewes of each breed x nutrition (total =24) combination were euthanized. Ewe blood samples were obtained immediately prior to slaughter and foetal samples by cardiac puncture immediately thereafter. Free amino acid concentrations in plasma were determined by HPLC using pre-column derivitisation with ortho-phthalaldehyde after precipitation of protein with sulphosalicylic acid. Effects of nutrition and breed were analysed in a 2x2 factorial analysis by ANOVA.

Results The most abundant (decreasing order of concentration) amino acids in maternal plasma were glycine, glutamine and alanine and in foetal plasma, glutamine, alanine and serine. After a period of re-feeding, differences in amino acid concentration ($\mu\text{mol/l}$) as a result of under nutrition were few and restricted to maternal glutamic acid (C v R, 44 v 65, s.e.d. 6.1, $P=0.004$) and glutamine (293 v 333, s.e.d.16.2, $P=0.023$) and foetal glycine (688 v 499, s.e.d. 89.6, $P=0.049$). However, the ratio of foetal to maternal amino acids was consistently greater in Suffolk than Blackface ewes with the differences being most marked for the acidic and branched chain amino acids (see Table 1).

Table 1 Ratio of foetal to maternal plasma free amino acid concentrations.

Amino acids	Scottish Blackface		Suffolk		<i>P</i> -value [†]	
	Control	Restricted	Control	Restricted	s.e.d.	Breed
Basic	0.83	0.94	0.99	0.92	0.128	NS
Acidic	2.82	1.95	4.74	3.74	0.509	0.002
Branched chain	2.09	1.83	2.59	2.51	0.196	0.009
Non essential	2.80	2.26	3.50	2.95	0.319	0.040
Essential	1.66	1.63	2.00	1.92	0.138	0.032
Total	2.23	1.94	2.74	2.37	0.219	0.043

[†] Effects of nutrition and breed x nutrition were not significant. s.e.d for 6 observations per mean.

Conclusions The absence of differences in plasma amino acid concentration as a result of under-nutrition is in agreement with Werkman et al. (2009) who concluded that the ability of the placenta to transport nutrients was not compromised by modest reductions in feed intake during the first 90 days of pregnancy. However, the higher foetal to maternal amino acid ratios for Suffolk ewes suggests a superior ability to transport amino acids which may be because of greater placental mass (Werkman et al. 2009) or placental efficiency.

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Effects of plane of nutrition and selenium supplementation of ewes in early and mid-pregnancy on meat quality of offspring

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Introduction Nutritional status in early and mid-pregnancy can affect subsequent offspring productivity. For example, plane of nutrition in early and mid-pregnancy has been shown to have a significant effect on carcass characteristics, with male offspring of dams offered a low plane diet in early pregnancy having poorer carcass conformation and greater fat depths over the muscle *L. dorsi* than lambs of dams offered medium or high plane diets (Muñoz *et al.* 2008a). Supplementation with selenium (Se) during this period had positive effects on measures of lamb viability and survival (Muñoz *et al.* 2008b). The aim of the current study was to investigate the effect of plane of nutrition and selenium supplementation of ewes in early and mid-pregnancy on the meat quality of the male offspring.

Materials and methods Between days 0 and 39 after synchronized mating (early pregnancy, EP) multiparous ewes (n = 99) were allowed 60% (low, L), 100% (medium, M) or 200% (high, H) of requirements for maintenance. Between days 40 and 90 (mid-pregnancy, MP), ewes were allowed either 80% (M) or 140% (H) of their maintenance requirement. Between days -14 and 90 post mating, ewes received Se treatments providing either no Se (Control) or 1 g of Selplex® = intake of 0.5 mg Se/ewe/day (Treated). After day 90 of gestation, all ewes were fed to meet energy and protein requirements for late pregnancy and supplemented with a standard multivitamin and mineral mix (Se content of 10 mg/kg). Male offspring (n = 73) were reared on a grass-based system and slaughtered at 42, 46 or 50 kg live weight (LW). Between ribs 6 and 12 the muscle *L. dorsi* was removed 24 h post-mortem for instrumental meat quality analyses. Measurements of pH, colour, cooking loss, Warner-Bratzler shear force (WBSF) and sarcomere length were recorded using the methods detailed by Moss *et al.* (1993). The data were analysed using the Genstat REML procedure in a 3 (EP plane of nutrition) X 2 (MP plane of nutrition) X 2 (Se supplementation) factorial design. The model examined the effects of the treatments, while adjusting for the effect of dam and sire breed, siblings and fat classification.

Results There were no statistically significant interactions between nutritional treatments, therefore, only main effects are presented (Table 1). Plane of nutrition in early and mid-pregnancy had no effect on any parameter of meat quality ($P > 0.05$). Selenium supplementation had a significant effect on meat colour with lambs from selenium-treated ewes having increased redness values (a^*) of the muscle compared with controls ($P < 0.05$). A similar tendency was observed for metric chroma values ($P = 0.072$). Selenium supplementation of ewes had no effects on the sarcomere length, ultimate pH, cooking loss or WBSF of male lambs ($P > 0.05$).

Table 1 Effect of plane of nutrition and selenium supplementation in early and mid pregnancy on meat quality of offspring

	Early pregnancy nutrition				Mid-pregnancy nutrition				Selenium supplementation		Significance [†]
	L	M	H	s.e.d.	M	H	s.e.d.	Control	Treated	s.e.d.	Selenium
Colour [‡]											
L*	42.0	39.8	40.9	2.700	40.5	41.3	2.189	42.2	39.6	2.155	
a*	11.5	11.4	13.2	1.329	12.4	11.7	1.060	11.0	13.0	1.044	*
b*	11.2	11.1	11.1	0.681	11.1	11.2	0.539	10.9	11.4	0.531	
Hue	45.6	44.7	40.8	3.296	42.5	44.9	2.617	45.5	41.9	2.579	
Chroma	16.3	16.0	17.4	1.252	16.8	16.4	0.998	15.7	17.5	0.983	P=0.072
Sarcomere length (mm)	1.78	1.78	1.76	0.029	1.77	1.78	0.023	1.76	1.78	0.023	
Ultimate pH	5.63	5.59	5.63	0.037	5.61	5.63	0.029	5.62	5.61	0.029	
Cooking loss (%)	23.2	22.8	22.8	1.235	23.3	22.6	0.989	22.7	23.2	0.971	
WBSF (kg/cm ²)	1.73	1.83	1.65	0.174	1.67	1.81	0.143	1.77	1.71	0.154	

[‡]Illuminant D65, 2 degree observer; L* lightness, a* redness, b* yellowness, Hue = $\tan^{-1}(b^*/a^*)$, Chroma = $(a^{*2}+b^{*2})^{0.5}$

[†]There was no statistical significance between early and mid-pregnancy treatments; * = $P < 0.05$.

Conclusion Selenium supplementation of ewes throughout pregnancy, compared with supplementation only in late pregnancy, improved meat appearance by maintaining the redness of the muscle. This could have implications in prolonging meat shelf-life. Plane of nutrition in early and mid-pregnancy of dams had no effects on offspring meat quality.

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Effect of lipid-rich plant extract on the fatty acids composition and meat quality of Charolais × Friesian steers

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Introduction Nutrition has a major impact on the fatty acid composition of beef, despite high levels of biohydrogenation of dietary polyunsaturated fatty acids (PUFA) in the rumen (Scollan *et al.*, 2006). The latter may be ameliorated by feeding rumen-protected lipid. Grass relative to concentrate feeding results in beef characterised by a higher content of *n*-3 PUFA and excellent colour shelf life. This study investigated the effects of including a PUFA-rich lucerne-based plant extract (PX) on the fatty acid composition and meat quality in beef.

Materials and methods Thirty Charolais × Friesian steers (~22 weeks of age) were allocated to one of three dietary treatments; 1) forage (grass/white clover swards during grazing followed by grass silage in winter period), 2) restricted barley straw and concentrate (40:60 on a DM basis; CONC), and 3) restricted barley straw and concentrate with “PX” (40:60 on a DM basis; PX-CONC). The “PX” is a PUFA-rich lucerne-based plant extract (Désialis – France Luzerne, France) developed from the liquid fraction extracted from fresh lucerne (*Medicago sativa* L.), and then heat-treated and dried. Liveweight was monitored every 28 days and gains were calculated for individual animals. This data was used to modify feed intake of the CONC and PX-CONC animals to achieve a similar growth rate to those fed on *ad libitum* forage. Animals were slaughtered when they achieved fat class 3L and samples of *longissimus thoracis et lumborum* were taken at 48 h post-mortem for fatty acid analysis, vitamin E analysis, 10-d aged samples for sensory analysis, or shelf life studies in modified atmosphere packs. An analysis of variance was conducted with diet as the main factor using GenStat (11th edition) statistical software.

Results Liveweight gain, carcass weight, conformation score and fatness score were similar across diets averaging 0.881 kg/d, 354 kg, 75.8 and 82.1, respectively. Total fatty acids and amounts of the major saturated fatty acids were not different (Table 1). The amount of 18:2*n*-6 and 18:3*n*-3 were higher on PX-CONC relative to either forage or CONC, resulting in higher P:S ratio on PX-CONC. Longer-chain PUFA (EPA and DHA) were up to three times higher (*P*<0.001) in forage-fed animals relative to the CONC. Vitamin E content of the muscle was higher (*P*<0.001) in animals offered forage whilst animals fed on CONC and PX-CONC were lower and less than the recommended 3.5 mg/100 g muscle required for optimum stability. This was reflected in lipid stability (TBARS, Table 1). Sensory quality was not different (Data not shown).

Table 1 Fatty acid composition (mg/100 g muscle) of *longissimus thoracis et lumborum*, and TBARS and vitamin E content of muscle in Charolais steers given experimental diets

	Forage	CONC	PX-CONC	SEM	P
Total fatty acids (mg/100 g muscle)	3461	3721	2960	0.10	NS
16:0	886	1012	756	0.12	NS
18:0	428	456	366	0.10	NS
18:1 <i>n</i> -9	1273	1256	959	0.10	NS
18:1 <i>trans</i> (t10 to 15)	41.0	74.5	45.0	0.14	0.012
CLA (<i>cis</i> -9, <i>trans</i> -11)	12.0	10.8	10.1	0.12	NS
18:2 <i>n</i> -6	60.6	120.1	147.3	0.05	< 0.001
18:3 <i>n</i> -3	32.0	10.2	48.9	0.09	<0.001
20:5 <i>n</i> -3 (EPA)	17.6	4.7	13.4	0.07	<0.001
22:6 <i>n</i> -3 (DHA)	4.18	1.03	1.93	0.064	<0.001
P:S ratio	0.07	0.08	0.16	0.080	<0.001
<i>n</i> -6: <i>n</i> -3 ratio	1.3	6.9	2.4	0.05	<0.001
Vitamin E (mg/kg muscle)	4.95	2.32	2.87	0.053	<0.001
TBARS, d 10 (mg/kg muscle)	1.1	3.4	5.7	0.16	<0.001

SEM is on log scale. TBARS = Thiobarbituric acid reactive substances

Conclusions Forage feeding increased the content of *n*-3 PUFA and shelf life of the meat. PX-CONC resulted in the highest level of total PUFA in meat relative to forage or CONC. This may be related to the rumen protected nature of the lipid in the concentrated plant extract. Additional vitamin E in the diet is required to increase stability of the meat from animals fed on both CONC and PX-CONC.

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Comparison of the performance of finishing beef cattle offered grass silage and legume / cereal wholecrop silage either alone or in combination with grass silage

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Introduction In Northern Ireland grass silage is the basal forage for beef cattle during the winter feeding period. Increasingly however, other ensiled forages are being considered due to their potentially lower cost and perceived conservation and nutritional attributes. Wholecrop cereals such as wheat have been shown to improve dry matter (DM) intake but not animal performance (Keady *et al*, 2007). With increasing volatility in world markets creating major fluctuations in protein prices, interest has increased in home grown protein sources. High protein legumes with low input costs, such as lupins have given reasonable animal performance when offered as a monoculture. However, limited scientific research has been undertaken on the role of legumes in combination with a cereal as a bi-crop for finishing beef cattle. Consequently the objective of the current study was to evaluate the effects of legume/cereal wholecrop offered either alone or in combination with grass silage on the performance of beef cattle.

Materials and methods Eighty continental cross steers with a mean initial live weight 557 ± 32 kg and mean age 18.5 ± 1.2 months were allocated to one of 10 treatments, balanced for genotype, initial weight and farm of origin. The five forage diets offered included solely grass silage (GS), lupins/triticale silage (L/T) and vetch/barley silage (V/B) offered either as the sole forage or in combination with GS at a ratio of 70:30, on a dry matter (DM) basis, legume/cereal wholecrop: GS. The forages were offered *ad libitum* once per day following mixing in a paddle type complete diet mixer wagon, supplemented with either 2 or 5 kg concentrates/head/day. Daily intakes and fortnightly chemical analysis of forages and concentrates were used to calculate supplementation level of soyabean meal within treatments to stabilise protein intake over all treatments. Equal numbers of steers from each treatment were slaughtered after 105, 119, 126 and 140 days on experiment according to liveweight, from heaviest to lightest. Data were analysed as a 5 forage x 2 concentrate level experiment with genotype, farm of origin, fat class and start weight included as covariates using GenStat REML.

Results Lupins/triticale, vetch/barley and grass silage had concentrations of dry matter, ammonia nitrogen, starch, crude protein (CP) and a pH of, 291, 304 and 251 g/kg fresh, 120, 140 and 90 g/kg total nitrogen, 112.3, 101.3 and 5.6 g/kg DM, 97, 147 and 122 g/kg DM and 4.04, 4.68 and 3.87 respectively. Grass silage had a predicted D-value of 680 g/kg DM. Total intake of crude protein (CP) of animals on 2 kg concentrates/head was stabilised at 1272 g/day and at 5 kg concentrates/head at 1505 g/day. There were no significant silage type by concentrate level interactions. Increasing concentrate supplementation from 2 kg to 5 kg/concentrate/head/day increased final liveweight (626 v 664 kg, sed 6.3, $P < 0.05$), liveweight gain (LWG) (0.59 v 0.87 , sed 0.054, $P < 0.01$), carcass weight (338 v 364 , sed 3.0, $P < 0.001$), and carcass gain (0.30 v 0.50 , sed 0.029, $P < 0.001$). The effects of forage type on feed intake and animal performance are presented in Table 1. Steers offered GS had significantly ($P < 0.001$) heavier final live weights compared to animals offered L/T, V/B and V/B:GS. Steers offered GS had significantly ($P < 0.001$) greater carcass weights and carcass gains compared to L/T, L/T:GS, V/B and V/B:GS. Animals offered GS had a similar silage dry matter intake (SDMI) to L/T and V/B although significantly ($P < 0.001$) lower than L/T:GS and V/B:GS.

Table 1 Effect of feeding grass silage, lupins/triticale and vetch barley as sole forages and in combination and concentrate level on feed intake and animal performance

	Forage (F)					sed	Sig.
	GS	L/T	L/T:GS	V/B	V/B:GS		
SDMI (kg/day)	5.79 ^a	5.98 ^{ab}	6.34 ^{bc}	5.84 ^a	6.19 ^{bc}	0.110	***
Final live weight (kg)	664 ^b	638 ^a	650 ^{ab}	631 ^a	643 ^a	9.4	***
LWG (kg/day)	0.92 ^c	0.67 ^{ab}	0.76 ^{bc}	0.59 ^a	0.69 ^{ab}	0.080	***
Carcass weight (kg)	366 ^b	347 ^a	353 ^a	344 ^a	348 ^a	4.5	***
Carcass gain (kg/day)	0.52 ^d	0.33 ^a	0.41 ^c	0.35 ^{ab}	0.38 ^{bc}	0.043	***
Dressing proportion (g/kg)	557	543	544	546	543	8.0	NS
Conformation class ^γ	3.4	3.4	3.4	3.2	3.1	0.17	NS

GS: Grass silage; L/T:GS: Lupins/triticale : Grass silage on a 70:30 DM ratio; L/T : Lupins/triticale; V/B : Vetch/Barley; V/B:GS: Vetch/Barley : Grass silage on a 70:30 DM ratio.

SDMI: Silage dry matter intake; Sig: Significance;

γ: EUROP scale : 5,4,3,2,1, respectively; LWG: Live weight gain;

Conclusion Finishing beef cattle offered legume/cereal wholecrop silage as the sole forage or in combination with grass silage had, on average, 26% lower liveweight gain relative to high quality grass silage. These results demonstrate that legume/cereal wholecrop silages do not offer a suitable alternative to high quality grass silage for finishing beef cattle.

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Effects of dietary protein concentration on the efficiency of nitrogen utilisation in lactating dairy cows

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Introduction The European Union Nitrates Directives set a limit on the amount (170 kg/ha) of manure nitrogen (N, = faecal N + urine N) that may be applied to land each year. This limit has 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 systems. The objectives of the present study were to evaluate the effects of dietary crude protein (CP) concentration on the efficiency of N utilisation in lactating dairy cows.

Materials and methods A large continuous design study (47 first and 40 multi-lactation Holstein dairy cows) was undertaken to examine effects of 3 dietary CP concentrations (180, 150 and 120 g/kg DM) on animal performance and nutrient utilisation from week 1 to 44 of lactation. The 3 mixed diets each contained 450 g/kg DM of forage (60% grass silage and 40% maize silage (DM basis)) and 550 g/kg DM of concentrates. Concentrate supplements ($n = 3$) consisted of different proportions of the same ingredients (barley, wheat, sugar beet pulp, citrus pulp, soya bean meal, rapeseed meal and molasses). Full details of experimental design, animal, diets and managements are reported by Law et al (2008). During early (70 to 90 days), mid (150 to 170 days) and late (230 to 250 days) lactation, the same 4 cows and 4 heifers from each treatment were transferred to metabolism units for 8 days, with measurements of feed intake and faeces and urine outputs during the final 6 days. Live weight was recorded at the beginning and end of digestibility measurements and milk yield and fat, protein and lactose concentration in milk were measured daily during the digestibility trials. The data were analysed as a one way ANOVA, with experimental period as block.

Results Dietary CP concentration had no significant effect on live weight or fat, protein or lactose concentration in milk, but increasing dietary CP concentration significantly increased DM intake (16.5, 18.4, 19.5 kg/d, s.e. 0.43, $P < 0.001$) and milk yield (20.6, 26.1 and 28.3 kg/d, s.e. 0.94, $P < 0.001$). Consequently, increasing dietary CP concentration significantly increased N intake, N outputs in faeces, urine and milk and N retention ($P < 0.001$, Table 1). Increasing dietary CP concentration significantly reduced N output in faeces and milk as a proportion of N intake ($P < 0.001$), while significantly increased urine N output ($P < 0.001$) and N retention ($P < 0.01$) as a proportion of N intake. There was no significant difference in milk N output as a proportion of N intake between low and medium CP diets. Manure N output as a proportion of N intake was significantly higher with low than medium and high CP diets ($P < 0.001$). However, manure N output as a proportion of DM intake or milk yield significantly increased with increasing dietary CP concentration ($P < 0.001$). There was no significant difference in manure N output as a proportion of milk yield between low and medium CP diets.

	Dietary CP concentration (g/kg DM)			s.e.	P value
	120	150	180		
Nitrogen intake (g/d)	321.5	444.6	561.8	10.53	< 0.001
Faecal N output (g/d)	134.9	161.9	172.9	5.14	< 0.001
Urine N output (g/d)	92.0	137.6	206.8	5.43	< 0.001
Milk N output (g/d)	99.5	132.3	143.9	4.35	< 0.001
Retained N (g/d)	-4.9	12.8	38.2	1.84	< 0.001
Faecal N/N intake (g/g)	0.425	0.363	0.308	0.0097	< 0.001
Urine N/N intake (g/g)	0.282	0.311	0.368	0.0082	< 0.001
Milk N/N intake (g/g)	0.310	0.297	0.256	0.0068	< 0.001
Retained N/N intake (g/g)	-0.017	0.030	0.068	0.0043	< 0.01
Manure N/N intake (g/g)	0.707	0.673	0.676	0.0065	< 0.001
Manure N/DM intake (g/kg)	13.8	16.3	19.5	0.19	< 0.001
Manure N/milk yield (g/kg)	11.4	12.0	14.1	0.53	< 0.001

Conclusions The results demonstrate that manure N output associated with one kg milk production increased with increasing dietary CP concentration, but the increase was not significant with diets containing CP levels above 150 g/kg DM. This indicates that overall dietary CP concentration of 150 g/kg DM may be appropriate in order to reduce manure N output, whilst reducing the decrease in milk yield normally observed with very low protein diets.

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An evaluation of the effect of nutritional strategy in early lactation on performance and energy status of Holstein Friesian dairy cows

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Introduction Improving the sustainability and overall longevity and health of modern Holstein Friesian (HF) dairy cattle is essential in order to maintain overall farm profitability. Prolonged negative energy balance in early lactation can result in metabolic stress, as well as long term problems with fertility (Pryce et al., 2004). In addition, maximising the efficiency of nitrogen (N) utilisation by strategies such as reducing the crude protein content of the diet is of crucial importance in minimising the environmental impact of dairying. The aim of this study was to evaluate the effect of a range of nutritional strategies on milk production, energy balance and efficiency of N use in early lactation.

Materials and methods Ninety six HF dairy cows were allocated to one of four dietary treatments immediately post calving. Multiparous animals (n=58) were balanced for milk yield in the previous lactation, parity (mean 2.4), liveweight and body condition score at calving. Primiparous animals (n=36) were balanced for liveweight and body condition score at calving. All diets were offered as a total mixed ration (TMR). The 'Control' diet was formulated using Feed into Milk (Thomas, 2004) to supply 200g starch /kg DM with an overall crude protein (CP) content of 175g/kg dry matter (DM). An 'Individual Cow Treatment' offered a basal TMR of 175g CP/kg DM. Dietary protein content was adjusted on the basis of calculated weekly energy balance, with protein content of the diet reduced to 150g/kg DM (if cows exceeded a pre-determined negative energy balance threshold) or increased to 200g CP/kg DM (if cows exceeded an upper energy balance threshold). A third treatment (High/Low Starch) involved offering a high starch diet for the first 50 days post partum (days 1-35 and 36-50 on 200 and 300 g starch/kg DM respectively). From day 51 to 120 post partum, animals were offered a low starch (100 g starch/kg DM) /high protected fat (37.5 g/kg DM, calcium salts of palm fatty acids) diet. The fourth treatment (Low Protein) was formulated to contain 150g CP/kg DM, with an additional supplement of 40g/head/day of protected methionine. All diets were offered *ad libitum* and contained concentrate and forage at a ratio of 60:40 with the forage component of the diet comprised of grass and maize silage (60:40). Individual feed intake was measured daily using Calan gates, milk yield and liveweight were also measured daily. Milk composition and condition score were measured weekly. Animals remained on treatment for the first 210 days of lactation. Data were analysed using REML available in Genstat and ANOVA.

Results Dry matter intake, milk yield, milk composition and liveweight change (from calving to 210 days) were not significantly different between treatments (all $P > 0.05$, Table 1). However, daily energy status was affected by treatment ($P < 0.05$) (Table 1), with the Individual cow treatment having the highest average daily energy status compared to the other three treatments (Table 1, Figure 1). In addition, efficiency of N utilisation was significantly different between treatments ($P < 0.001$) with the low protein diet having the most efficient utilisation of N.

Table 1 Treatment effects on animal performance for the first 210 days of lactation

	Control	Indiv. cow	High/Low starch	Low protein	S.E.D. ¹	Significance
Dry matter intake (kg/day)	19.8	20.6	19.7	19.6	0.67	ns
Milk yield (kg/day)	33.5	33.7	34.7	33.4	1.63	ns
Milk composition						
Fat (g/kg)	38.1	39.2	38.4	38.5	1.38	ns
Protein (g/kg)	34.0	34.0	33.8	33.8	0.50	ns
Liveweight change (kg/day, calving-210 days)	0.20	0.14	0.15	0.16	0.06	ns
Milk N/N intake (kg)	0.313 ^b	0.285 ^c	0.336 ^b	0.376 ^a	0.02	***
Daily energy status (MJ/day)	15.4 ^b	28.7 ^a	16.8 ^b	11.9 ^b	5.86	*

¹S.E.D., standard error of the difference. ² Significance, ns, $P > 0.05$; *, $P < 0.05$; ***, $P < 0.001$

Figure 1 Daily energy status (first 210 days of lactation)

Conclusion Manipulation of individual cow diets in early lactation may help to maintain daily energy status. Reducing the crude protein content of the diet, improved N utilisation with no significant reduction on milk yield or effect on milk composition, however daily energy status was reduced.

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Manipulating the fatty acid composition of lipids in the digital cushion of the bovine claw

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Introduction The fatty acid (FA) composition of animal tissues is influenced by many factors including age, dietary forage to concentrate ratio, breed and energy status. Despite a high degree of bio-hydrogenation of unsaturated FA's in the rumen, another crucial influence on tissue FA composition is the FA composition of the diet. Feeding linseed has been shown to increase omega-3 (n-3) polyunsaturated fatty acids (PUFA) in beef. Altering the fat content and composition of the bovine digital cushion has potential implications for lameness in cattle. This study aimed to test the hypothesis that cattle offered extruded linseed would show a marked difference in FA composition in the lipids of the digital cushion compared to cattle offered a diet with no linseed.

Materials and methods Entire male cattle were offered a grass silage/concentrate-based diet balanced for energy. In each treatment, a proportion of the concentrate was substituted for a linseed supplement (Valomega 160 BCC, Premium Crops Ltd; comprised of 0.70 Linseed TRADI-LIN® and 0.30 wheat bran) at zero, 400, 800, or 1200g per head per day for an average of 140 ± 27 days during the finishing period. Target live weight at slaughter was 540kg. Animals were slaughtered in 3 batches after 120, 147 and 185 days on experiment. Digital cushions were dissected in 6 sections (A-F, Räber et al., 2006) from the right lateral hind claws. Lipids were extracted and expressed as a proportion of the fresh tissue, saponified and converted to Fatty Acid Methyl Esters (FAME). The FA distribution was determined by gas chromatography (HP6890) using a 25m column with helium as carrier gas. FA were expressed as % of FA present. Data were analysed by Analysis of Variance using Genstat 10.1 (Lawes Agricultural Trust, 2007) with diet, location (A-F) and their interactions as fixed effects and fat content (g/100 g tissue) as a covariate. Main effects and linear or quadratic contrasts were examined.

Results Total lipid content varied by sample location (A-F; $P < 0.05$) but not diet. The effect of dietary treatment on the concentration of a selection of FA's is shown in Table 1. Although the proportion of C18:3n-3 was not significantly different across treatments, the concentration of C18:3n-3 as a proportion of total PUFA was significantly greater in animals offered 1200 g linseed supplement/day relative to the other treatments ($P = 0.01$). A linear relationship was observed between an increasing level of linseed supplementation and the concentration of 18:3n-3/total PUFA (positive relationship; $P = 0.003$) and the n6/n3 ratio (inverse relationship; $P = 0.04$). Many fatty acids showed a quadratic relationship with level of linseed supplementation. For example, the proportion of monounsaturated FA (MUFA) was higher in the 0 and 1200 g/day treatments ($P = 0.002$), whereas the PUFA's were lower in the 0 and 1200 g/day treatments compared to the intermediate treatments ($P = 0.002$). Irrespective of treatment differences, the relatively high proportion of MUFA overall in the digital cushion is likely driven by function not diet, since previous studies have shown much higher levels of MUFA in the digital cushion compared to perirenal adipose tissue (Räber et al., 2006).

Table 1 Effect of level of linseed supplementation on proportion of individual fatty acids (g FA/ 100 g FA) in the digital cushion and the ratio of various fatty acids. (MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; SFA = saturated fatty acid)

Fatty Acid (g FA/100 g FA)	Level of linseed supplement (g/head/d)				Avg s.e.	Main effect	Linear	Quadratic
	0	400	800	1200				
C18:2n-6	2.05	2.87	2.35	2.06	0.22	0.03	NS	0.01
C18:3n-3	0.25	0.32	0.29	0.33	0.03	NS	NS	NS
MUFA	77.28	75.82	75.80	77.65	0.50	0.01	NS	0.002
PUFA	3.16	4.98	4.15	3.46	0.40	0.01	NS	0.002
PUFAn-6	2.63	3.99	3.26	2.75	0.32	0.01	NS	0.004
PUFAn-3	0.54	0.99	0.89	0.72	0.09	0.01	NS	0.001
C18	69.05	71.07	70.82	69.08	0.88	NS	NS	0.03
n-3:total PUFA	0.17	0.23	0.24	0.23	0.02	NS	0.03	NS
18:3n-3:total PUFA	0.09	0.09	0.09	0.13	0.01	0.01	0.003	NS
PUFA:SFA	0.16	0.26	0.21	0.19	0.02	0.01	NS	0.004
n6:n3	7.33	3.66	3.31	3.74	1.17	NS	0.04	NS

Conclusions This work demonstrates the influence of dietary fat on the type of fat deposited in the digital cushion. There appears to be an optimal level of supplementation of linseed, after which, the deposition of PUFA's in the digital cushion decreases, as seen here in the 1200g/d treatment. Potential to manipulate the composition of the digital cushion could have important ramifications for lameness in cattle.

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The effect of dairy cow breed and cross on milk production and fatty acid composition under grazing conditions

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Introduction Dietary intake of omega-3 polyunsaturated fatty acids (n-3 PUFA) and conjugated linoleic acid (CLA) has been recognized as having potential positive effects on human health (Lock and Bauman, 2004). Furthermore, alteration of the fatty acid (FA) composition of milk through targeted manipulation of dairy cow diets can be achieved (Dewhurst et al., 2006). Although breed effects on the FA composition of milk have previously been reported (Kelsey et al., 2003), most work to-date has been conducted under high input TMR based systems. Furthermore, little is known on the effects of heterosis on concentrations of FA in milk. The objective of this study was to examine whether differences in milk FA concentration exist between two contrasting breeds of dairy cow and their F₁ hybrid managed under a grazing based production system.

Materials and methods Eighty-one spring-calving dairy cows (27 Holstein Friesian, 27 Jersey and 27 Holstein X Jersey cross) with a mean $\pm$ SD calving date of February 12th $\pm$ 16.4 d were used. Cows were managed under a predominantly perennial ryegrass (*Lolium perenne* L.) pasture. Two experimental periods were evaluated (June and July). Individual milk yields (kg) were recorded daily and milk fat, protein and lactose concentrations were recorded on one successive evening and morning, one day per week. Milk fat was extracted and methylated using an adaptation of the method reported by Christie (1982). Data were analyzed by ANOVA and mean differences were separated using the PDIF command of SAS (adjusted by Tukey test). Cow was used as a random effect and the statistical model include terms for breed, period and its interaction.

Results There was no interaction for any of the variables studied and thus data were pooled across the two periods. Holstein Friesian cows had the highest milk yield, Jersey the lowest with the crossbred animals intermediate ($P < 0.01$). Conversely, milk fat and protein were highest for Jersey, lowest for Holstein and the crossbred animals intermediate ($P < 0.01$). Differences were found for n3:n6 ratio ($P < 0.05$), C18:2 n6, and the $\Delta 9$ desaturase index ($P < 0.01$) with all higher for Holstein animals. CLA tended to be higher in Holstein possibly as a consequence of a higher $\Delta 9$ desaturase activity (Table 1).

Table 1 Effect of breed on milk production and milk fatty acid composition

Item	Holstein	Jersey	Holstein x Jersey	SED ¹	Significance
Milk production (kg/d)	21.1 ^a	14.5 ^c	17.3 ^b	0.40	<0.01
Milk fat (%)	3.79 ^c	5.09 ^a	4.45 ^b	0.130	<0.01
Milk protein (%)	3.43 ^c	4.01 ^a	3.76 ^b	0.054	<0.01
Fatty acids (g/kg of Total Fatty Acids)					
C18:0	79.7	83.0	81.6	3.60	0.67
cis-9 C18:1	154.6	147.7	148.5	10.20	0.82
trans-11 C18:1	44.4	45.2	38.4	3.95	0.30
cis-9 cis-12 C18:2 n6	5.9 ^a	4.7 ^b	4.6 ^b	0.38	<0.01
cis-9 trans-11 C18:2 (CLA)	17.8	15.3	14.7	1.36	0.08
C18:3 n3	6.6	6.0	6.6	0.35	0.16
n3	6.9	6.2	6.7	0.38	0.21
n3:n6	0.77 ^b	0.99 ^a	1.01 ^a	0.100	0.03
$\Delta 9$ desaturase index ²	0.09 ^a	0.08 ^b	0.08 ^b	0.004	<0.01

^{a, b}Means within a row with different superscripts differs ($P < 0.05$); ¹Standard error of the differences;

²C14:1/(C14:0 + C14:1).

Conclusion The results of this study show that under the grazing conditions employed here, Holstein dairy cows produced more milk with a higher content of CLA potentially due to a higher activity of the $\Delta 9$ desaturase enzyme in mammary gland. There was no evidence for heterotic effects on any milk related variables measured.

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The effect of a short extended grazing period in the spring and straw allocation on the milk yield and composition of autumn calving dairy cows

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Introduction Milk production from grass by autumn calving dairy cows can be increased through extending the grazing season in early spring, therefore reducing the reliance on more expensive conserved forage and concentrates (Peyraud *et al.*, 2004). Turning cows out to pasture earlier in the spring has also been associated with an increase in milk and milk protein yield (Sayers and Mayne, 2001). In contrast, milk fat content may be decreased, possibly due to the lower fibre content of spring grass (Sayers and Mayne, 2001). The inclusion of chopped straw in the ration offers the potential of increasing dietary fibre intake and therefore maintaining milk fat levels (Ferris *et al.*, 2000). The objectives of the current study were to determine the effect of extending the grazing season in spring and increasing the fibre content of the ration on the intake and performance of autumn calving dairy cows.

Materials and method Thirty six Holstein-Friesian dairy cows (nine primiparous and 27 multiparous) that were approximately 180 d into lactation were randomly allocated to one of 3 dietary treatments based on parity, stage of lactation and performance in the week prior to allocation. The control group (C) received a total mixed ration (TMR) containing (kg/kg; DM basis) 0.34 maize silage, 0.18 grass silage, 0.15 caustic treated triticale, 0.09 soyabean meal, 0.06 rapeseed meal, 0.08 dairy compound, 0.06 sugarbeet pulp, 0.035 molasses and 0.005 minerals/vitamins. The ration was fed once daily at 0900 h at 1.05 of ad-libitum intake through Insentec roughage intake feeders (Insentec, Marknesse, The Netherlands). The two grazing groups received both the control TMR ad-libitum and 2 h/d grazing (CG) or the control TMR with an additional 1 kg/cow/d of chopped straw mixed with the ration, along with 2 h/d grazing (CGS). Cows in treatments CG and CGS were restricted from the TMR from 0500 h until turnout each day, at approximately 0800 h and were set-stocked on a 1.2 ha perennial ryegrass sward that had last been grazed at the end of November 2007. When not grazing, all animals were housed in the same section of a cubicle house. Grazing commenced on 5th March 2008 and the study lasted for 3 wks. Milk yield was recorded at each milking and samples taken for subsequent analysis during week 0, mid way through the study and at the end of week 3. Body weight and condition score were measured at the beginning and end of the study. Grass samples were taken from 3 random 25 x 35 cm areas of the paddock at 4 cm above ground level during weeks 1, 2 and 3 of the study for subsequent analysis. Data were analysed as a randomised block design using Genstat 10.1 (VSN Int. Ltd., Oxford, UK).

Results The average grass DM was 221 g/kg, whilst crude protein and neutral detergent fibre were both high at 211 and 530 g/kg DM respectively. There was no effect ($P>0.05$) of access to grazing or straw allocation on milk yield, composition or component yield (Table 1). In contrast, cows that received access to pasture consumed on average 5.05 kg DM/d less than those that received the TMR alone. There was no effect of treatment on live weight or body condition score change.

Table 1 Effect of a control diet (C), access to grazing for 2 h/d (CG) or access to grazing for 2 h/d and additional straw provision (CGS) on the performance and TMR intake in dairy cows during a 3 week period.

	C	CG	CGS	s.e.d.	P-value
Milk yield (kg/d)	31.9	32.0	31.4	0.862	0.727
Fat (g/kg)	42.7	42.0	41.6	0.18	0.824
Fat (kg/d)	1.34	1.33	1.30	0.060	0.796
True protein (g/kg)	32.7	31.4	32.0	0.09	0.324
True protein (kg/d)	1.02	0.99	1.00	0.028	0.614
Weight change (kg/d)	1.12	0.44	0.27	0.445	0.158
CS change	-0.02	0.02	-0.12	0.105	0.374
TMR DM intake (kg/d)	20.9	16.8	14.9	2.99	<0.001

Conclusions Allowing access to grazing for 2 h/d in the spring had no effect on milk yield or composition in autumn calving dairy cows, but resulted in a reduction in TMR intake of 5 kg DM/d. There was no effect of inclusion of 1 kg/cow/d of straw on milk fat content in cows that received access to grazing, possibly due to the relatively high fibre levels in the over-wintered grass.

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Physical effectiveness of ensiled apple pomace and its effects on rumen passage rate of digesta in Holstein dairy cows

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Introduction Apple pomace (AP) is the by-product obtained when apples are pressed to make juice and is produced in huge (97,000 tons/year) amounts in Iran. It's characterized by low starch and high NDF concentrations and accompanied by appreciable quantities of poorly identified components, including soluble carbohydrates and pectin. Optimal utilisation of diets by dairy cows is influenced by the chemical composition and physical characteristics of the ration. Physical characteristics can influence animal health, rumen fermentation and utilisation, animal metabolism, and milk fat production independently of the amount or composition of chemically measured components (Mertens, 1997). Water holding capacity may affect rumen liquid pool size and the rate and extent of rumen digestion (Allen and Mertens, 1988). The purposes of the current study were to determine the physical effectiveness of ensiled apple pomace (EAP) and its effects on passage rate from the rumen of Holstein dairy cows.

Materials and methods AP was obtained from a factory in Urmia city and stored in a 30 tons trench silo for ensiling. The silo was sealed for 45 days. This experiment was carried out at the dairy barn of Urmia university, Iran. The effects of EAP on rumen passage rate of digesta were measured using three multiparous lactating Holstein dairy cows (body weight = 565 ± 16 kg; days in milk = 75 ± 23) in a 3×3 (three periods and three diets) Latin square change over experiment. Cows were held in individual tie stalls during this experiment, and were fed ad libitum twice daily as a total mixed ration at 0700 and 1900 h, to provide approximately 100 gr/kg refusals each day (as-fed basis). Cows had free access to water 24 h/day. The first 14 days of each period were used for dietary adaptation and the last seven days for measurements. Diets had similar chemical composition and were formulated using NRC (1989) and AFRC (1995) recommendations. Diets consisted of 46% forage and 54% concentrate on DM basis including lucerne hay, barley grain, cotton seed meal, EAP, sodium bicarbonate, dicalcium phosphate, vitamin and mineral premix, and salt. The treatments were control diet no EAP, 150 gr/kg EAP and 300 gr/kg EAP DM basis. EAP substitute instead of lucerne hay in the diets. Samples of EAP were prepared by milling oven dried EAP (fine), through a 2-mm screen of miller for measuring bulk density, water-holding capacity and functional specific gravity. Bulk density (g/ml), insoluble dry matter (g/kg of DM), and water-holding capacity (g/g insoluble DM) of EAP were measured according to Giger-Reverdin (2000). Functional specific gravity and hydration rate of EAP was measured using a 100-ml pycnometer at 39.0 ± 0.5 °C (Wattiaux, 1990). Chromium-mordanted NDF of straw as a single dose marker, were prepared and used to measure rumen particulate passage rate and retention time (Uden et al., 1980). Marker administration, sampling procedures and use of markers to estimate passage rates carry out following the procedure described by Osuji et al (1993). Data were analysed using the PROC GLM of SAS (1998) and the NLIN procedures of SAS (1998).

Results Water-holding capacity, functional specific gravity, hydration rate, insoluble DM, bulk density of EAP were 4.487(g/g insoluble DM), 1.263, 0.042 (g/g insoluble DM /hour), 730.8 (g/kg of DM), and $0.553 \text{ (g/cm}^3\text{)}$ respectively. Rumen passage rate of digesta in Holstein dairy cows was significantly affected by substitution of EAP (Table 1). Rumen passage rate of digesta decreased with increased inclusion of EAP in the diet. Control diet (no EAP) had a higher rumen passage rate and lower rumen retention time than others.

Table 1 Rumen passage rate of digesta in Holstein dairy cows

	Diet			s.e.m	P
	Control	150 g/kg EAP	300 g/kg EAP		
Rumen passage rate (%/hour)	7.34 ^a	7.01 ^b	6.36 ^c	0.003	0.0001
Rumen mean retention time (hour)	13.62 ^c	14.26 ^b	15.72 ^a	0.003	0.0001

Conclusions The physical effectiveness of EAP may describe rumen passage rate and rumen mean retention time. According to results, high water-holding capacity and low bulk density of EAP is result of high pectin content and high proportion of cell wall content. Otherwise, as the proportion of silage was increased rumen particulate passage rate and retention time significantly decreased and increased respectively. Higher water-holding capacity and high pectin content of pomace silage reduce rumen passage rate and increase rumen retention time of digesta.

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An investigation of calf rearing practices on dairy farms in Northern Ireland

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Introduction Without a detailed understanding of how calf rearing is performed in practice, it is difficult to establish 1) the effectiveness of technology transfer initiatives and 2) how best to focus future research efforts. In view of this, the objective of the current study was to investigate calf rearing practices on Northern Ireland dairy farms.

Materials and methods A stratified random sample of 300 dairy farms was selected from the Northern Ireland population of 3252 farms with 30 or more dairy cows in June 2005, (Northern Ireland Agricultural Census). The sample was stratified by county and dairy herd size (30-50, 51-80 and 80 plus dairy cows) and selected with equal proportion of farms from each group, to ensure good representation within the sample. Only farms with dairy heifer rearing enterprises were included. In total 247 farmers participated in an interview-based questionnaire. SPSS software was used to carry out statistical analysis. Statistical association tests were carried out including non-parametric statistical tests and contingency table tests using Chi Square tests.

Results Calves were reared by the farm owner on 62% of farms, with a family member having primary responsibility on 36% of farms. Bull calves were reared for beef enterprises on 35% of farms, whilst 58% of farms sold bull calves at 1-2 weeks of age, with 4% of farms selling bulls at weaning. On most farms (73%) colostrum was mainly supplied by suckling from the dam. As herd size increased, the calf was more likely to remain with the dam for a shorter period of time ($P < 0.01$). Indeed, for every unit increase in herd size, the likelihood of the calf remaining with mother for >24 hours reduced by a factor of 0.011 (OR (odds ratio) = 0.989; CI (confidence interval) = 0.982-0.997; $P < 0.01$). Colostrum was administered to every calf via oesophageal feeder on 24% of farms and this practice was more likely to occur as farm size increased (OR = 1.013; 95% CI = 1.007-1.019; $P < 0.001$). During the pre-weaning phase, calves were most commonly housed in individual pens (48%) with larger farms more likely to use group pens ($P < 0.05$) and group feeding systems ($P < 0.001$). However, twice daily bucket feeding was the dominant technique (79% of farms). Within calf accommodation, ventilation was based on natural airflow on 93% of farms. However, there was a significant herd size effect, with smaller farms less likely to use forced ventilation (OR = 0.992; 95% CI = 0.984-0.999; $P < 0.05$). Milk replacer was used predominantly on 23% of farms, with 77% of producers offering whole milk, of which 58% of producers used milk destined for the bulk milk tank. Calf starter concentrate was made available to calves pre-weaning on 98% of farms, with 88% offering greater than 1kg/day during the week immediately prior to weaning. The delayed introduction of calf starter post-birth was associated with an increased age at first calving ($P < 0.001$). Straw and hay were the most popular forages offered at birth (60 and 30% of farms respectively), during the milk feeding stage (59 and 33% of farms respectively) and at weaning (46 and 28% of farms respectively), with grass silage gaining popularity only at weaning (25% of farms). Although the mean weaning age was 8 weeks, 28% of producers weaned at greater than 8 weeks (Figure 1). The findings from this study suggest that calf size was the primary criterion used to determine when to wean dairy calves (Table 1).

Table 1 Relative importance of weaning criteria

Weaning Criteria	Percentage of farms rated factor important or greater
Size	89.4
Solid food intake	80.3
Age	65.3
Date	16.5
To empty pen/house	15.5

Figure 1 Weaning age profile of dairy calves

Conclusion These results demonstrate that a wide range of practices have been adopted for rearing dairy calves in Northern Ireland. Increasing herd size was associated with a number of rearing practices that potentially reduce labour requirements. However results indicate considerable opportunity exists for improved efficiency highlighted by 28% of producers weaning calves at greater than 8 weeks. This information will enable focused research to address potential inefficiencies and enable more targeted technology transfer.

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Effects of level and form of dietary zinc on dairy cattle performance and keratin production in the teat canal

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Introduction The teat canal in dairy cows is lined with keratin, which is considered to be a natural physical and chemical barrier involved in providing resistance to bacteria gaining entry into the mammary gland. Keratin is partially removed during milking therefore requiring regeneration. It has been estimated that a cow needs to regenerate 1.3 mg per teat between each milking to replace the amount lost (Capuco *et al.*, 1992). Zinc is important in the process of keratinisation and previous studies have reported an 2.4 kg/d increase in milk yield when supplemented with Zn in an organic form at the recommended level and an increase in somatic cell counts (SCC) in cattle receiving 0.66 of the recommended level of dietary Zn (Atkin *et al.*, 2007), although mechanisms for this observation are unclear. The objective of this study was to establish the effect of level and form of Zn (organically chelated (Bioplex ZnTM; Alltech Inc., Nicholasville, USA) vs. inorganic (ZnO)) and no supplemental Zn on dairy cow performance and keratin production in the teat canal.

Materials and method A total mixed ration containing (DM basis, kg/kg) 0.19 maize silage, 0.19 grass silage, 0.19 whole-crop wheat silage, 0.09 caustic wheat, 0.08 soyabean meal, 0.08 rapeseed meal, 0.07 molassed sugar beet pulp, 0.05 molasses, 0.03 maize gluten feed, 0.02 fat and 0.01 minerals (without zinc) was offered at 1.05 of *ad libitum* intake. The basal diet was predicted to supply 792 mg Zn/d and was supplemented with one of five concentrates differing in their level and form of dietary Zn. The concentrates were formulated to provide an additional 600 mg Zn/d (to supply the total recommended level, according to NRC (2001), in an inorganic (RI) or organically chelated form (RO)), or 396 mg Zn/d (to supply 0.86 of the total recommended level in an organically chelated form, O86) or 198 mg Zn/d (to supply 0.72 of the total recommended level in an organically chelated form, O72), or no extra supplemental Zn (C), and were fed at the rate of 2 kg/cow/day in one meal through out of parlour feeders. Fifty multiparous Holstein-Friesian dairy cows, that were on average 27 days (s.d. +/- 8.4) into lactation were allocated to one of the five treatments. Milk yield and feed intake were recorded daily. The technique for keratin sampling was based on that of Capuco *et al.* (1990) and was carried out prior to the evening milking during weeks 0, 5 and 10 of the experiment from both hind quarters. Additional samples were taken from the cows in week 10 to determine the regeneration of keratin at 0, 10, 24, 48 and 72 hours post sampling. Statistical analysis was conducted using GenStat version 10 (VSN Int. Ltd., Oxford, UK).

Results Mean total DM intake and mean milk yield were 24.9 kg/d and 41.8 kg/d respectively, but neither differed ($P > 0.05$) between treatments (Table 1). The amount of keratin recovered did not differ ($P > 0.05$) between treatments over the 10 week period or 0 hours to 72 hours collection. Keratin regeneration was estimated to be 1.4 mg of fresh weight in 24 hours. There was no effect ($P > 0.05$) of dietary treatment on Zn concentration of keratin at any time point (Table 1). SCC were not significantly affected by treatments, however initial SCC were low ($< 190,000$ cells/ml) and declined during the study (mean end value of 89,000 cells/ml).

Table 1 Effect of level and form of dietary Zn on keratin production and Zn concentration from cows fed diets that contained either the recommended level of inorganic (RI) or organic (RO) Zn, 0.86 of the recommended level of organic (O86), 0.72 of the recommended level of organic (O72) or no supplemental Zn (C).

	Treatments					s.e.m	P-value
	RI	RO	O86	O72	C		
Total DM intake (kg/d)	24.0	25.0	24.9	26.2	24.7	0.76	0.387
Milk yield (kg/d)	41.2	42.7	42.8	41.9	40.4	1.239	0.515
Teat keratin (fresh weight (mg/teat) (0-10 weeks)	6.8	6.8	8.3	7.0	7.5	0.441	0.104
Teat keratin (fresh weight (mg/teat) (0-72 hours)	5.4	5.6	6.5	5.6	6.3	0.410	0.200
Zn concentration of keratin (mg/kg DM) (0-10 weeks)	1.8	1.7	1.8	1.7	1.7	0.138	0.571
Zn concentration of keratin (mg/kg DM) (0-72 hours)	2.2	2.0	1.9	2.0	2.0	0.192	0.218

Conclusions Keratin regeneration and Zn concentration of keratin was not significantly affected by Zn level or form in this study. The previously reported increase in milk yield from the organic form at the recommended level and the decrease in SCC from the recommended level of Zn supplementation may not therefore be related to an effect on teat keratin production.

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Prediction of metabolisable energy concentration of fresh grass – which digestibility variable is better, organic matter or energy?

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Introduction Accurate prediction of the ME concentration of fresh grass is vital in developing appropriate feeding regimes for grazing animals. While AFRC (1993) proposed an equation to calculate grass ME concentration from digestible OM in total DM (DOMD), the accuracy of this approach has been questioned (Yan and Agnew, 2004). The objective of the present study was to develop a prediction method for ME concentration of fresh grass from nutrient digestibility.

Materials and methods Fresh herbage was harvested daily at 13.00 h for a seven week period (from early growth through to late maturity) from the primary growth of a perennial ryegrass sward. Herbage was offered to two groups of dry, non-pregnant dairy cows at maintenance level of energy intake. Fresh herbage was offered twice daily, with one portion offered at 14.00 h and the other stored at 4 ° C and offered at 09.00 h the following day. The first group of four cows was on treatment for four weeks, with total collection of faeces and urine from weeks two to four. During the fourth week, grass for the first group was also offered to the second group of four cows, and faeces and urine collected for the second group for the last three days. Measurements with the second group of cows continued until the end of week seven. A similar procedure was repeated for the first regrowth from a second sward and for the second regrowth from a third sward. Grass ME concentration was calculated using methane energy output, predicted from an equation developed using the same dataset presented in the present study (Yan and Mayne, 2008). Three-day mean data ($n = 37$) were used to develop prediction equations for ME concentration using nutrient digestibility and DE concentration.

Results and discussion Data obtained during week four (overlap period) were used to evaluate the effect of cow group. There was no significant difference in nutrient digestibility between the two groups for each sward. As expected, increasing grass maturity from week two to seven increased ADF (0.212 to 0.298) and NDF (0.449 to 0.550) concentrations (kg/kg DM), while reducing CP (0.151 to 0.066) and lipid (0.038 to 0.016) concentrations (kg/kg DM). Consequently, DOMD (0.795 to 0.703 kg/kg DM), DE (15.2 to 12.9 MJ/kg DM) and ME (12.5 to 10.3 MJ/kg DM) concentrations reduced from week two to seven. A range of linear regression equations have been developed to predict ME concentration from nutrient digestibility and DE concentration (Table 1 and Figure 1). The relationship between ME concentration and DM digestibility (Eq. [1]) was relatively poor, while R^2 increased and s.e. reduced when OM digestibility and DOMD were used (Eq. [2] and [3]). When the constant was omitted, ME concentration was 15.47 MJ/kg of DOMD (Eq.[4]). This value is slightly lower than that (16.0) recommended in AFRC (1993). However, the R^2 increased considerably (0.86 or 0.91) and the s.e. reduced (0.26 or 0.22) when GE digestibility or DE concentration (Eq. [5] or [6]) were used. The lower R^2 with DOMD prediction, when compared to DE concentration, may be due to the fact that DOMD does not take account of the variation in energy content with different digestible nutrients (lipid, protein and carbohydrates).

Table 1. Prediction equations for ME content #			
Equations	R^2	s.e.	Eq.
$MEc = 12.51_{(1.98)} DMd + 1.96_{(1.57)}$	0.53	0.49	[1]
$15.56_{(1.86)} OMd - 1.0_{(1.55)}$	0.67	0.41	[2]
$21.29_{(2.03)} DOMD - 4.5_{(1.56)}$	0.76	0.35	[3]
$15.47_{(0.082)} DOMD$	0.70	0.39	[4]
$17.86_{(1.20)} GE_d - 2.4_{(0.96)}$	0.86	0.26	[5]
$0.935_{(0.051)} DEc - 1.7_{(0.74)}$	0.91	0.22	[6]
# MEc or DEc = ME or DE concentration (MJ/kg DM)			
DMd, OMd, GE _d or DOMD = DM, OM, GE digestibility			
or digestible OM in total DM (kg or MJ/kg DM)

Fig 1. Relationship between ME content and DOMD

Conclusions A range of linear regression equations have been developed to predict ME concentration using nutrient digestibility and DE concentration in fresh grass. The strongest relationships were with GE digestibility or DE concentration.

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Effects of normal and high oleic acid rapeseed in the dairy cow diet on milk fatty acid composition

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Introduction Milk and dairy products are the major source of saturated fatty acids (SFA) in the European diet and there is evidence that replacing SFA with *cis*-monounsaturated fatty acids (MUFA) will improve risk factors for cardiovascular disease. Rapeseed in dairy cow diets simultaneously reduces milk SFA and increases *cis*-MUFA, but also increases milk *trans* MUFA. A new variety of rapeseed with higher oleic acid content (therefore less potential for increasing milk *trans*-MUFA) was compared with a normal oleic acid rapeseed, and both compared with a control diet.

Materials and methods Seven early lactation multiparous Holstein-Friesian cows were randomly allocated to one of seven total mixed ration diets (50:50 forage:concentrate) for 5 x 28 day periods, in an almost Balanced Incomplete Block Design. The forage proportion of the diets contained both maize and grass silages (3:1). A control diet [C] contained 41 g/kg dry matter of calcium salts of palm oil fatty acids, and six treatment diets contained one of two different rapeseeds (normal oleic acid content [NOR], Mozart and Astrid varieties, and high oleic acid content [HOR], Nexera variety) milled with wheat (750 kg wheat to 1000 kg whole rapeseed) and included at three inclusion levels so that diets provided approximate intakes of 750, 1000 and 1250 g/cow/day rapeseed oil. Diets were formulated to be isoenergetic and isonitrogenous. Representative samples of feed constituents (including both rapeseed/wheat mixes) were collected for fatty acid analysis. Milk samples from each cow were collected from the last two milkings of each period, combined according to yield and stored frozen prior to fatty acid analysis. Milk and feed fatty acids were determined using the method outlined by Kliem *et al.* (in press). Milk fatty acids were expressed as g/100 g total fatty acids. Data were analysed using a general linear model with effects for cow, period and treatment. Statistical significance of the following were measured: (1) control vs NOR inclusion level 1, (2) control vs HOR inclusion level 1, (3) linear response of NOR, (4) linear response of HOR, (5) average NOR vs average HOR.

Results Oleic acid content of the HOR/wheat mix was 70 g/100 g total fatty acids compared with the NOR mix which was 58 g/100 g total fatty acids. A higher oleic acid content meant that the HOR mix had a lower 18:2 *n*-6 and 18:3 *n*-3 content (16 and 2.0 g/100 g total fatty acids respectively, compared with 20 and 8.2 g/100 g total fatty acids respectively for NOR mix). Including both rapeseeds (level 1) decreased ($P < 0.01$) the proportion of milk SFA (Tables 1 & 2), increased ($P < 0.05$) milk total *cis*-MUFA and *trans*-MUFA. The SFA and *cis*-MUFA effects were linear ($P < 0.001$) with increasing inclusion level for both rapeseeds, and the effects were greater for the HOR diets than for the NOR diets ($P < 0.01$). Despite numerically displaying a linear response, the milk *trans*-MUFA effect was not statistically linear with increasing inclusion level. Surprisingly, there was also no significant difference between the two different rapeseed types for milk *trans*-MUFA.

Table 1 Effect of rapeseed type and inclusion level on milk fatty acid composition (g/100 g total fatty acids)

	Control	NOR rapeseed			HOR rapeseed			SEM
		Level 1	Level 2	Level 3	Level 1	Level 2	Level 3	
SFA	66.5	63.3	61.0	58.5	61.2	60.4	56.6	0.55
<i>cis</i> -MUFA	25.1	27.1	29.1	31.1	29.3	30.0	33.1	0.46
<i>trans</i> -MUFA	4.2	5.8	6.2	6.7	5.8	6.0	6.9	0.41

Table 2 Statistical significance of (1) control vs NOR level 1, (2) control vs HOR level 1, (3) linear response of NOR, (4) linear response of HOR, (5) average NOR vs average HOR (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS - non significant).

	1	2	3	4	5
SFA	**	***	***	***	**
<i>cis</i> -MUFA	*	***	***	***	***
<i>trans</i> -MUFA	*	*	NS	NS	NS

Conclusions The results show that both rapeseed supplements were effective at lowering milk SFA and increasing *cis*-MUFA linearly, and that the HOR rapeseed was more effective at doing this, probably due to the higher oleic acid content. However, despite having a lower polyunsaturated fatty acid (PUFA) content, the HOR diets caused a similar increase in milk *trans*-MUFA to the NOR diets, suggesting that the variation in fatty acid profile between the two rapeseeds was insufficient to influence ruminal production of *trans*-MUFA. A rapeseed with a higher oleic acid content (and therefore lower PUFA content) may have had a greater influence, or perhaps a higher inclusion level.

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Effect of dairy management (conventional, organic) and season (winter, summer) on fatty acid composition of retail whole milk

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Introduction Milk fat is characterised by the complexity of its components both in biosynthesis and structure. It has often been linked with negative effects on human health although some milk fatty acids (FA) have been found to have a beneficial effect on human health (German and Dillard, 2006). Saturated fatty acids (SFA) have been associated with the increase of risk of cardiovascular heart disease, high concentrations of LDL-cholesterol and insulin resistance. However, milk also contains unsaturated fats both monounsaturated (MUFA) and polyunsaturated (PUFA) which have been reported to benefit human health (Givens and Shingfield, 2004). PUFA can be further categorized as ω -3 and ω -6 FA. The main ω -3 FA in milk is α -linolenic acid (ALA) and the main ω -6 FA in milk is linoleic acid (LA) both of which are important, since they cannot be synthesised in mammals and have to be supplied from dietary origins (Ruxton et al., 2005). Other beneficial FA in milk include cis-9,trans-11 conjugated linoleic acid (c9t11 CLA) and trans-11 C18:1 (VA), a precursor of CLA. PUFAs have been shown to be involved with protection against coronary heart disease, prevention of cancer, neurological functions, brain and visual development on infants and antiobesity (Shingfield et al., 2005). The aim of this study was to investigate the differences in fatty acid composition between organic and conventional commercial whole milk during winter and summer seasons.

Materials and methods In this survey, 36 different sources of commercial whole milk, characterised by brand and management system, were collected from retail outlets in the North East of England, 11 brands were certified and labelled as organic. Milk purchase was carried out over two years on 4 sampling dates: 2 during winter (January 2007, January 2008) and 2 during summer (August 2006, August 2007), providing 114 samples in total. Analysis of FA methyl esters was performed with a Gas Chromatography system (Shimadzu, GC-2014, Japan) using a Varian CP-SIL 88 fused silica capillary column (100m x 0.25mmID x 0.2 μ m film thickness). Peaks were identified using a 39 FAME and c9,t11CLA isomer standards. Analysis of variance (ANOVA) using general linear model (GLM) was used to analyze results in Minitab 15 using "Management system" (conventional or organic) and "season" (winter or summer) as factors.

Results Table 1 shows organic milk contained significantly higher concentrations of all beneficial FA compared to conventional milk and summer milk was significantly higher in VA, c9t11 CLA and α LN but lower in LA than winter milk. Table 2 shows that organic management significantly increased PUFA and had no effect on SFA and MUFA concentration of milk. Summer milk had significantly higher concentrations of PUFA and MUFA and lower SFA content than winter milk.

Table 1 Relative proportions (%) of individual fatty acids in organic and summer milk compared with conventional and winter milk respectively

	Organic	P-value	Summer	P-value
VA	+33.3%	***	+104.4%	***
CLA	+27.0%	***	+75.6%	***
α LN	+55.1%	***	+40.1%	***
LA	+12.5%	***	-5.4%	**

Significances were declared at ***: P<0.001, **: P<0.01

Table 2 Relative proportions (%) of fatty acid groups in organic and summer milk compared with conventional and winter milk respectively

	Organic	P-value	Summer	P-value
SFA	-0.8%	NS	-6.5%	***
MUFA	-0.5%	NS	+16.1%	***
PUFA	+20.3%	***	+14.6%	***

Significances were declared at ***: P<0.001, NS: P>0.05

Conclusions Milk produced either under organic management or during August is likely to have higher concentrations of specific unsaturated fatty acids shown to be beneficial for human health compared with milk produced under conventional management or during January respectively. Concentrations of all beneficial FA were found higher in organic and summer milk, except the case of LA on summer milk and both factors also had an overall beneficial influence on milk fatty acid group profiles.

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Use of selenium enriched fertilizers in the management of a suckling herd: effects over a 5 year period

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Introduction Antioxidant mechanisms, immune responses, reproduction, thyroid metabolism are processes in which the trace element selenium (Se) is involved. In beef production with suckling herds, a Se deficiency could impair animal health resulting in reduced incomes. In Belgium the Se content in locally produced feedstuffs is low and thus, in beef farms where locally produced feedstuffs are fed for the entire year, symptoms of Se deficiency are observed. The aim of the present study was to assess the effect, of Se enriched fertilizers on the Se content of feedstuffs and on the Se status of cattle.

Material and methods Se enriched fertilizers were used since 2002 on the Experimental Research Station of the Veterinary Faculty of the University of Liege. For the grazing pastures, 3 g of Se/ha was applied at each nitrogen application. For pastures used for grass silage 3g of Se/ha was applied at the beginning of the season and then after each cut. Four g Se/ha were spread at the 2nd and 3rd nitrogen applications for barley production. For maize from year 3, 8 g Se/ha was applied at sowing. There were control pastures – fields with no Se application but similar management. The Belgium Blue suckling herd was divided in 2 at the beginning of the trial with about 18 cows in each group. Cows, calves and young stock remained in their respective group throughout the experiment. During the grazing period, animals had only access to grazed pastures. During the winter period, the ration was made of grass silage, maize silage and barley produced with or without Se enriched fertilizers as appropriate to treatment group and supplemented with dehydrated lucerne, sugar beet pulp and bran.

Results The changes in plasma Se and in Se in the red blood cells along with the dietary Se content are given in Figure 1. The average dietary Se was 54, 247 and 138 µg/kg DM in the control group and in the Se groups during summer and winter respectively. At the beginning of the trial, plasma Se concentration was low for both groups. It remained low during the 5 years of the trial in the control group. By contrast, the plasma Se content was increased in the Se group by the end of the first winter period. A further increase was observed during the grazing season. The following winter was characterized by slightly lower concentrations. Similar patterns were observed during the following grazing and winter periods. The Se content in the red blood cells followed a similar pattern to that observed for plasma. The reduced concentrations during the winter periods as compared to grazing periods were due to reduced Se intake associated with the purchased feedstuffs offered during the indoors period.

Conclusion It is concluded that the use of Se enriched fertilizers can improve and maintain a high Se status in a beef herd managed over many years on locally produced feedstuffs during the succeeding outdoors and indoors seasons.

Figure 1 Changes according to the different seasons of the total plasma Se concentrations (figure 1a), of the Se content in the erythrocytes (Figure 1b) measured by the glutathione peroxidase activity and of the dietary Se contents (Figure 1c) of Belgian Blue cattle managed in a production system with (light grey) or without (dark grey) Se enriched fertilizers.

***In vitro* degradability of different fractions of *Solanum lycocarpum* St Hil.**

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Introduction Naturally grown in the vast majority of Brazilian territory, *Solanum lycocarpum* (SL, popularly known as wolf's apple) can be used as alternative feed additive for ruminants. Despite its significant amount of secondary metabolites, it is known to be eaten by cattle and a specific species of wild wolf (*Chrysocyon brachyurus*). However the effects of these organic compounds on ruminal fermentation are still unclear and a dose-response is expected to turn a potential feedstock into an undesirable baneful aliment (Goel *et al.*, 2008). As a result scientific investigation regarding this highly promising plant is needed to test its potential as a supplement to support sustainable livestock production systems. Thus this study compared the effects of different dried meal-like fractions (fruit, leaf, flower, root, stem) of this plant at two different levels (0.2 and 0.4 g) on the *in vitro* dry matter (DM) degradability (IVD) at three different times of incubation.

Materials and methods Samples of SL were collected from Brazilian region at Lavras, fractionated, freeze-dried, ground and transported by air to Newcastle. A triplicate 5x2x3 factorial experiment measured the effect of these five dried meal-like fractions of SL (Flower=Fl, Fruit=Fr, Leaf=Lf, Stem=St and Root=Rt) at two levels (0.2 and 0.4g of DM) on the *in vitro* degradability at three distinct incubation times (08, 24 and 48h). Samples of either 0.2 or 0.4 g dried ground SL were separately weighed into 50 ml centrifuge tubes to which 40 ml of the inoculum was added under CO₂. Rumen fluid (RF) was obtained at Cockle Park Farm at Newcastle University from two fistulated sheep before morning feeding (0900 h) and transported in warm-insulated flasks under anaerobic conditions to the laboratory. Subsequently RF was pooled and strained through cheese cloth into pre-warmed flasks. Then it was mixed in a 50 ml polypropylene centrifuge tube with the pre-warmed buffer at 1:4 ratio to prepare inoculum. RF was then immediately added. Tubes were flushed with CO₂ to ensure anaerobic conditions and sealed with rubber stoppers containing pressure release valves and incubated in water bath (WB) at 39°C for the pre-determined times. After each incubation time the tubes were removed from the WB, stoppers removed, screw capped immediately and submerged in ice to stop fermentation and allow accurate operational procedures. The liquids and residues were separated by centrifuging the tubes at 3000 rpm for 10 minutes. Residues were washed with distilled water using sintered glass crucibles under vacuum. The residues were dried at 60°C for 48h to determine DM degradability by using equation $IVD = 1 - \text{Undegraded Residue (UR)} \text{ (g/kg)}$. The IVD data were analysed using the General Linear Model of Minitab to compare the main effects of SL fractions and the two levels as well as their interaction on the IVD at each incubation time.

Results The mean IVD for each treatment combination is shown in Table 1. The main effect of SL fractions was significant for IVD at each time ($P < 0.001$) whereas the effect of SL level was not significant ($P > 0.05$). IVD of the fruit was the highest perhaps due to the greater amount of soluble carbohydrates (Figure 1) than those of flower and leaf and lowest for Rt and St. No significant interaction between SL and Level was observed ($P > 0.05$). As a result IVD of SL fractions were not dose-dependant in this study.

Table 1 Mean IVD of *Solanum lycocarpum* fractions at 2 different levels and 3 incubation times (%)¹

Fraction/Inc. Time / Level	8 h		24 h		48 h	
	0.2	0.4	0.2	0.4	0.2	0.4
Fruit	59 ^a	56 ^a	61 ^a	54 ^a	72 ^a	68 ^a
Flower	33 ^a	29 ^b	47 ^a	42 ^a	51 ^a	49 ^a
Leaf	11 ^b	12 ^c	22 ^b	23 ^b	42 ^b	43 ^b
Root	13 ^b	14 ^c	20 ^b	21 ^b	25 ^b	27 ^b
Stem	6 ^c	7 ^d	4 ^c	3 ^c	6 ^c	5 ^c
SEM	3.2	3.1	4.2	3.7	4.1	3.9

¹Means with different superscripts within a column are significantly different ($P > 0.001$)

Figure 1 Box plot of the IVD of *Solanum lycocarpum* fractions with respective means and std deviations

Conclusions IVD of the different fractions varied in-between but not within the levels of *Solanum lycocarpum* at each incubation time. It can be an indication that the release of secondary metabolites by fermentation breakdown was variable between fractions due to their different IVD. Characterization of main secondary metabolites as well as their effects on fermentation profiles at different incubation times will be progressed to recognize which approach must be taken to utilise this plant as a ruminant feedstock.

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Effect of yeast and mannan-oligosaccharides on *in vitro* fermentation of different substrates

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Introduction Results of yeast effects on *in vitro* rumen fermentation are inconsistent (Sullivan et al., 1999; Yang et al., 2004). In addition, there is no data on the effect of mannan-oligosaccharides (MOS) and their interaction with yeast on rumen fermentation. This trial was conducted to study the effects of yeast and MOS on rumen fermentation of different substrates.

Materials and methods A batch culture study was conducted using a completely randomised design with factorial arrangement. Treatments included two forage:concentrate ratios $\times$ five additives $\times$ three substrates. Ratios included either 60:40 (HL) or 40:60 (HB) of lucerne hay: barley grain fed to four ruminally fistulated sheep. Additives were: 1) no additive (NC), 2) monensin (5 ppm per fermentation medium) as a positive control (MON), 3) MOS (Agrimor[®]; 0.001 g), 4) live yeast (Levucell[®]; 3.5×10^5 cfu; SC), and 5) Mixture of MOS and SC (MOS+SC). Substrates were; 1) lucerne hay, 2) maize silage, and 3) barley grain. Amounts of SC and MOS were calculated according to product label for 0.5g substrate. The study was conducted in two consecutive periods (20 days) with HL fed in period 1 and HB fed in period 2 and rumen fluid was collected at the end of each period. Hence, rumen fluid taken from sheep in each period represented the effect of forage: concentrate ratio on fermentation parameters. Rumen fluids were combined prior to incubation and diluted with buffer solution with a ratio of 1:4 under anaerobic conditions. Fermentation continued for 24 h in 100 ml serum bottles containing 50 ml of inoculum and 0.5 g of substrate ground to pass a 1-mm screen. All treatments had three replicates. The pH, ammonia nitrogen, and *in vitro* dry matter disappearance (IVDMD) were determined upon opening. Gas production was recorded at 2 h intervals during the entire 24 h. Data were analysed by GLM procedure of SAS (SAS Institute, 1996). Treatment least square means were compared using PDIFF statement when the treatment effect in the statistical model approached significance ($p < 0.05$).

Results Among fermentation parameters, IVDMD and gas production were not affected by the interaction of ratio $\times$ additive $\times$ substrate. Interaction of ratio $\times$ additive on IVDMD, pH and ammonia nitrogen was highly significant indicating that the impact of additive changed when the nature of basal diet was changed. In the HL ratio, the SC increased total gas production (102.1 vs. 83.8 ml, SE=4.19) and decreased pH (6.30 vs. 6.41, SE=0.008) in the lucerne samples compare to NC. The MOS+SC also decreased pH. Other additives had no significant effects on measured parameters. In the maize silage samples, MOS and MOS+SC significantly increased ammonia nitrogen in compare to NC (41.86 and 45.91 vs. 37.41 mg/100 ml, SE=1.791) and decreased pH. When substrate was barley grain, SC increased ammonia nitrogen (34.18 vs. 22.02 mg/100 ml, SE=1.791) and decreased IVDMD in compare to NC. Also MOS+SC increased ammonia nitrogen and pH relative to NC (38.9 vs. 22.02 mg/100 ml, SE=1.791 and 5.95 vs. 5.91, SE=0.009 for ammonia nitrogen and pH, respectively). Other parameters were not affected by MOS, SC or MOS+SC. Supplementation of MON generally decreased or did not change measured parameters. In the HB ratio, MOS and SC significantly increased total gas production in the lucerne samples compared to NC ($p < 0.05$). SC and MOS+SC increased ammonia nitrogen in compare to NC. In the maize silage samples; MOS and MOS+SC increased IVDMD in compare to NC (45.5 and 50.0 vs. 39.1 g/100 g, SE=0.02). The SC and MOS+SC decreased pH (6.21, 6.19 vs. 6.25 in NC, SE=0.008), while total gas production increased significantly by SC (80.96 vs. 67.3 ml, SE=4.66). With the barley grain samples, supplementation with MOS increased (5.89 ± 0.009) and MOS+SC decreased (5.82 ± 0.009) pH in compare to NC (5.85 ± 0.007). The SC and MOS+SC significantly decreased IVDMD (53.6, 50.1 vs. 60.1 g/100 g, SE=0.02). The MON supplementation decreased IVDMD in lucerne and maize silage but increased it in the barley samples. Ammonia nitrogen increased in the maize silage samples and gas production decreased in the lucerne and maize silage samples when MON was supplemented. Use of MON increased pH in the lucerne and maize silage samples ($p < 0.05$).

Conclusions Source of inoculum changed the measured parameters of substrate fermentation when they incubated with various additives. Selecting additives with the aim of improving digestibility should be based on the level of concentrate in the diet. Also, maximum benefit from using additives is obtained when substrate type is taken into consideration. The combination of SC and MOS had positive effect only on some parameters.

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Fermentation kinetic, chemical composition, organic matter degradation and metabolisable energy of three non-cultivated pastures located in the central plateau of Mexico

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Introduction Approximately 80% of all production costs are related to the purchase of forages and other feeds in dairy enterprises in Mexico. Resource poor, smallholder (*campesino*) systems are heavily reliant on the use of maize stover, non-cultivated/cultivated grass species and weeds to feed their dairy cattle, thus a nutritional understanding of local forage resources would be of considerable economic benefit. The objective of this study was to determine the nutritive value of non-cultivated pastures (NcP) across the growing season.

Materials and methods Three NcP designated as 1, 2 and 3 of which two were multi-species (1 and 2) and one dominated by kikuyu grass (*Penisetum clandestinum*) (3), located at the Toluca valley in the central highlands of Mexico (19° 27' N and 99° 38' W), were selected for study. Thirty individual samples were collected from each pasture per month for 12 months from summer 2006 to end of Spring 2007 (i.e. total samples per pasture = 360). The thirty individual samples from each pasture were combined into one for its later lab analysis. All standing plant material within a 0.50 m² quadrant was cut 5 cm above ground level and immediately dried at 60°C until constant weight. The sampling area was marked with pegs in order to ensure that samples were taken from the same area on each occasion. Plant samples were analysed for dry matter (DM) and crude protein (AOAC, 1990), neutral and acid detergent fibre (NDF and ADF) according to Van Soest *et al.* (1991). Metabolisable energy (ME) from organic matter degradation (OMd) (AFRC, 1995), OMd was obtained using gas production (GP) technique (Mauricio *et al.*, 1999). Rumen liquor was collected just before the morning feeding from two Friesian dairy cows fed 9.4 kg DM grass silage and 9 kg DM concentrate. Three replicates per substrate were used to measure GP and additionally four blanks (anaerobic medium and rumen fluid alone) were included. Rate of fermentation of organic matter (F_rOM) was obtained multiplying the gas released (ml) at each incubation time by the total OM degraded at 48 h (mg), then divided by the total GP at 48 h (ml), and the result expressed as a percentage. An ANOVA with repeated measurements was performed using Genstat[®] v15, with seasons (S) and hours post inoculation (t) as factors and pastures (P) as blocks.

Results Table 1 shows that seasons had a significant (P<0.001) effect on chemical composition and F_rOM. Organic matter degradation, CP, ME and the F_rOM decreased while DM and NDF content increased from summer to winter in pastures 2 and 3. Dry matter content increased from summer to spring in pasture 1.

Table 1 Chemical composition, OM degradation and F_rOM (/h)

Pt	Pn	Season	DM g/kg	OMd g/ kg DM	CP	NDF	ME MJ/ kg DM	F _r OM		
								8	24	48
Multi-species	1	spring	570	399	90	789	5.7	0.018	0.020	0.022
		summer	320	489	109	743	7.1	0.022	0.024	0.021
		autumn	276	397	84	790	5.7	0.021	0.024	0.020
		winter	430	412	87	790	6	0.019	0.020	0.022
Multi-species	2	spring	435	524	103	756	7.7	0.017	0.025	0.021
		summer	315	559	90	697	8.3	0.020	0.026	0.021
		autumn	440	487	74	767	7.2	0.019	0.026	0.019
		winter	740	461	63	793	6.8	0.015	0.022	0.022
Mono-species	3	spring	280	556	216	746	8.4	0.020	0.030	0.017
		summer	229	591	159	749	8.9	0.024	0.033	0.019
		autumn	389	556	159	740	8.4	0.026	0.033	0.017
		winter	724	481	113	760	7	0.024	0.029	0.017
		s.e.m.	49	19	13	8	0.3	0.0005	0.001	0.0018
		S	***	***	***	***	***	t	***	***
		P	***	***	***	***	***	t x S	***	***
		S x P	***	***	***	***	***	t x P	***	***

Pt pasture type; Pn number of pasture; s.e.m. standard error of the mean; *** (P<0.001)

Conclusions Summer and autumn are the seasons in which a higher F_rOM was observed in all pastures. Pasture 3 had higher F_rOM values than pasture 1 and 2. Chemical composition and ME concentration is influenced by time of the year (i.e. season) and pasture type. In this study the forage material from pasture 3 had a higher nutritional value than pastures 1 or 2.

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Effect of processing of palm oil petiole on palatability in Bali cows (*Bos sondaecus*)

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Introduction Palm oil petiole (POP) is a pruning by-product from palm oil plantations. POP is the stalk of the palm oil frond (POF) without the leaflets and the outer layer. By weight, it contributed about 30 percent of POF. POP was a possible alternative for POF in feeding Bali cows (*Bos sondaecus*) since Bali cows we observed to reject fresh POF. Central Bureau of Indonesian Statistics (2006) reported that the production of POF was around 10,869,365 t from around 3,682,900 ha of palm oil plantation in Indonesia which is equivalent to 3,260,810 t of POP. POF are successfully used as feed for *Bos taurus* and *Bos indicus* and there are a lot of studies being done, especially in Malaysia. Alimon and Hair Bejo (1995) reported that the chemical composition of POF is 47, 385, 787, 556 g kg⁻¹ and 5.65 MJ kg⁻¹ for crude protein (CP), crude fibre, neutral detergent fibre (NDF), acid detergent fibre (ADF) and metabolisable energy respectively. However, there is no information on the nutritive value of POP and its palatability in Bali cows. The aims of this study were to evaluate the nutritive value of POP, and palatability of POP types by Bali cows when processed in several forms.

Materials and methods Four two-year old Bali cows weighing around 112-122 kg were used in this study. Cows were placed in 2 x 2 m individual pens equipped with water and four feed containers. The four feed containers were meant for four different type of feeds offered to the cows. Fresh POP was obtained from a palm oil plantation. The palatability test followed the method created by Kryazakis (1993) in which all cows were offered four alternative feeds (treatments) namely field grass (native grass) as control (A), freshly chopped POP (B), POP meal (C) and POP pellet (D). B was prepared by freshly peeling and chopping of POP, C was prepared by oven drying of freshly chopped POP at 60 °C for 24 h followed by grinding, and D was prepared by mixing of rice bran and POP meal with ratio of 1:1 and pelleted to produce pellets of 0.5 cm diameter. Dustiness of feed D was reduced by moistening it with water. All the feeds were analysed for their nutrient contents (Table 1). At the adaptation period of four weeks, the four different feeds were alternately provided to the cows at one time for a week. Following the conditioning period, all the four feeds were made available to the cows in the four separate containers. At the commencement of the trial, cows had free access to all four treatments and water. Feeds were added twice a day at 0700 and 1700h. The values measured included the amount of DM consumed for each treatment. DM consumed was calculated based on the total amount of feed given minus the total amount of feed refusal in each feed container. The design of the study was Randomized Block Design (4x4) with four treatments and four blocks of cow as replications. Statistical analysis was done using anova and Duncan test (Steel and Torry, 1991)

Results There was a significant ($P < 0.05$) difference in consumption among the treatments (Table 2). The consumption of A and D were higher than B and C. This might be due to field grass being a conventional feed, usually eaten by the cows and D containing rice bran that may provide typical taste, form, texture and aroma that is accepted by the cows. Low consumption of B and C might be due to the readily availability of A and D. Feed type D was the most chosen feed among the four treatments with 42.9 % eaten by the animals. Nutrient contents of POP on dry matter basis are 51.4, 573.9, 384.9 g kg⁻¹ for CP, NDF and ADF respectively.

Table 1 Chemical composition of each treatment diet

Treatment	DM	Ash	CP	CF	EE
A	24.40	3.10	2.80	11.40	0.30
B	31.29	3.57	2.15	35.66	0.75
C	83.92	3.57	2.15	35.66	0.75
D	68.75	2.80	4.89	25.62	2.54

Table 2 DM consumption and DM proportion consumed for each treatment

Treatment	Consumption (gDM/h/d)	Proportion consumed (%)
A	1,772.48 ^a	54.22
B	61.65 ^b	1.88
C	32.27 ^b	1.00
D	1,402.48 ^a	42.90
Total	3,268.48	100.00

Conclusion It can be concluded that POP, around 30 % of OPF, could be utilized as feed. POP could be chopped, ground and pelleted. Pelleted POP (D) was the most preferred feed by Bali cows with the proportion of 42.9 % being eaten.

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The behaviour of cows in response to rubber mats in milking parlour stalls

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Introduction High-yielding dairy cows are at risk of injuries causing lameness due to the hard, abrasive nature or slipperiness of the usual concrete floor surface (Cook et al., 2004). These problems can be exacerbated by the milking procedure that includes crowding of cows and possible sharp turns at the entrance and exit of the parlour. Rubber mats on walkways have been shown to reduce injury (Rushen and Passillé, 2006) and are preferred by cows to a concrete or slatted surface (Telezhenko et al., 2007). A recent installation of rubber matting in the milking stalls of a 15 cows per side (30-30) rapid exit parlour provided an opportunity to observe potential effects on cow behaviour. The mats were introduced to one side at a time and then to both sides and the aim was to determine whether the rubber matting had any significant effects on social interaction immediately prior to entering the milking stalls and the time taken for 15 cows to take their places.

Materials and methods 526 Holstein cows (average yield >10 000 litres) from a single, zero-grazed dairy herd milked 3 times per day were observed. Rubber mats (11m x 1m x 1.58 cm) were installed to cover the main alley of both milking parlour sides (i.e. covering main walking alley when entering and under hind legs when in milking position). The observation included four periods: Week 1 – without mats (NOMAT); Weeks 2, 6, 7 – mat on the left hand side, only (LEFT); Weeks 8, 11, 12 – mats on the right hand side, only (RIGHT); Week 13 – mats on both sides (BOTH). Data were only recorded from the morning and afternoon milkings, which included all cows. There was a total of 2883 observations of cows filling a single side; 164 of these contained less than the maximum 15 cows and 1531 driven up by the herds person and were discarded leaving 1188 observations for analysis. The number of head butts and pushes during each side fill were recorded between the front two animals funnelling into the milking stalls. Head butt and pushing data were analysed by period using the Kruskal-Wallis test and differences between pairs of periods identified using the Mann Whitney U-test. Filling the parlour stalls was calculated as the mean of the time taken from the first cow entering the gates to the moment of taking position in stalls by the last (15th) cow. These data were transformed ($\ln(x)$) to normalise the data and then analysed using ANOVA with LSD tests for comparison between means of the different periods and by t-test to assess overall left or right-hand side preference. All statistics were carried out using Genstat for Windows (version 10).

Results The presence of mats on both sides of the milking parlour (BOTH) was associated with both increased head butts (Figure 1) and pushing among the cows (data not shown) by approximately twofold, compared with the other periods with either no mats or a mat on the left- or right-hand side ($p < 0.05$). There was a definite right-side preference overall with cows being generally quicker to fill that side (178.0 vs. 137.8 ± 1.03 s, left vs. right back-transformed means and SED, respectively, $p < 0.001$). There was also a significant decrease in time for the cows to fill the parlour side with mats present on both sides compared with either no mats present, present only on the left or only on the right side (Figure 2). The difference in time required to fill parlour stalls between the NOMAT period and the BOTH period was about 40 seconds per side.

Figure 1 Number of butts between cows (boxplot shows median & interquartile range, min. & max. values)

Figure 2 Time taken to fill milking parlour side (original means and SEM. P-value refers to transformed values)

Conclusion The installation of rubber mats was associated with a decrease in the time taken to fill the milking parlour and also with an increase in head butts and pushing between cows immediately waiting to enter the stalls. This might reflect an increased motivation to enter stalls when mats were present, which would indicate cow preference in addition to significant management benefits associated with reduced milking time. Limitations in experimental design mean that latency to fill side results should be treated with caution due to a possible generalised effect reducing over time.

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The prevalence of hock lesions in UK dairy cattle and the significance of risk factors associated with the development of the condition

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Introduction The term hock lesion describes incidences of hair loss, thickened skin, wounds, scabs, and swellings occurring over the tarsal joint. Hock lesions in dairy cattle are a widespread problem around the world. In the UK, a recent study found approximately 99% of examined cows had at least some callused area over a hock (Haskell et al., 2006). The presence of hock lesions is correlated with injuries of the teat, carpal joints and skin, as well as increases in mobility score, high somatic cell counts, a greater incidence of clinical mastitis, and higher culling and annual death rates. Differences in the prevalence and severity of hock lesions according to the lying surface and cubicle design have been identified. Cow-related factors and management variables have also been postulated as possible risk factors. Recognition of the condition and practical solutions aimed at resolving it have however, been limited. The aim of this study was to determine the prevalence of hock lesions in lactating cattle on dairy farms in the East Midlands of the UK, and the significance of potential risk factors in the development of the lesions.

Materials and methods A random sample of fifty lactating cows on each of 77 dairy farms in the East Midlands were assessed during a single day visit to each farm over the winter housing period of 2007/2008. The extent and severity of lesions over all aspects of the hock were assessed and recorded using a hock map. Hock lesions were additionally scored on a four-point scale for hair loss, ulceration, and swelling based upon scales described by Huxley and Whay (2006). Assessments were made of mobility score, body condition score, cleanliness, and rising behaviour. Days in milk, milk yield, age and parity data were collected. Measurements related to the animals' environment were taken, including dimensions of loafing areas and cubicles, quality of the lying surface, and the quantity of bedding material provided. Interviews were conducted with the farmers in an attempt to establish management characteristics and attitudes that may be driving the development of this condition.

Results and conclusion Analysis of the results is currently ongoing. Hock lesion prevalence data is shown in Table 1. The median percentages of hocks across all herds showing any degree of hair loss, ulceration, and swelling were 89.7%, 11.8%, and 100% respectively. The median percentage of hocks displaying hair loss, ulceration, and swelling of score 2 or above were 37.0%, 4.6% and 21.8% respectively. This paper will present data on the location, severity and prevalence of hock lesions in UK dairy cattle and identify factors and practices associated with the presence of the condition.

Table 1 Median, inter-quartile range and range of hock lesion scores from random samples of 50 cows on 76 UK dairy herds

Measure	Median (%)	Inter-quartile range (%)	Range (%)
Hair loss			
score 0	10.3	5.1-25.4	0.0-78.4
score 1	42.0	30.5-58.6	6.3-93.8
score 2	24.7	14.4-35.2	1.0-53.5
score 3	10.9	1.9-18.9	0.0-54.3
Ulceration			
score 0	88.2	72.8-94.5	48.9-100.0
score 1	6.5	3.8-12.4	0.0-34.0
score 2	4.2	1.1-7.5	0.0-23.4
score 3	1.0	0.0-2.9	0.0-15.6
Swelling			
score 0	0.0	0.0-0.0	0.0-7.3
score 1	78.2	70.4-89.0	35.3-100.0
score 2	19.0	10.6-28.0	0.0-55.9
score 3	1.0	0.0-2.9	0.0-14.0

Detailed hock score data and a full analysis of the associated risk factors will aid understanding of the potential mechanisms involved in the development of lesions, and provide practical methods for reducing the prevalence and severity of hock lesions in dairy cattle in the UK.

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Spatial distribution of Scottish Blackface ewes in comparison to other crossbreds on a hill environment

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Introduction Domestic sheep have a strong social tendency (Dwyer and Lawrence, 1999). Sheep recognise members of their own flock, and social bonds can determine grazing distribution. The distribution and distance between grazing ewes can vary widely depending on availability and quality of vegetation. However it tends to be a characteristic of breed (Sibbald and Hooper, 2003). Hill sheep breeds are known to have a higher flexibility than lowland breeds (Dwyer and Lawrence, 1999) and tend to have less need to be close to other animals in free ranging environments. The aim of this study was to monitor the behaviour and grazing patterns of a traditional upland breed, Scottish Blackface ewes in comparison to a range of other crossbred ewes.

Materials and methods A research programme evaluating the performance of Scottish Blackface (BxB), Swaledale X Blackface (SWXB), Cheviot X Blackface (CHXB), Lleyn X Blackface (LLXB) and Texel X Blackface (TXB) ewes was undertaken on six hill farms across Northern Ireland (Speijers *et al.*, 2007). In the current study four of these farms were selected to undergo ewe grazing patterns and behaviour monitoring. Monitoring took place during the grazing season of June to October 2007. Four ewes of each breed were marked with a different colour to ensure accurate identification on the hill. The ewes were returned to the hill with the rest of the flock and remained there throughout the grazing season. Observations were carried out weekly by walking the hill and observing the ewes from various vantage points on the hill. Time of observation ranged from 10am to 3pm. On each visit to the farm, ewe location was recorded. The locations were added onto a corresponding farm map in ArcView GIS 3.2. Distances between all of the marked ewes within each breed were measured in meters using the ArcView programme, and recorded on each day of observations. A total of 15 observations per ewe per farm were recorded and compatible for analysis. Data were analysed by analysis of variance using Genstat. The analysis compared maximum, minimum and average distanced between marked ewes within breeds, across the four farms for each observation, (weekly).

Table 1 Average distance between marked ewes within breed (meters)

Observation	BXB	SWXB	CHXB	LLXB	TXB	SEM	P-Value
1	349 ^a	258 ^b	216 ^b	115 ^c	123 ^c	52	<0.036
2	399 ^a	281 ^b	306 ^b	95 ^c	144 ^c	57	<0.017
3	300 ^a	231 ^b	223 ^b	152 ^c	178 ^c	31	<0.05
4	481 ^a	230 ^{bc}	239 ^b	221 ^{bc}	191 ^c	45	<0.004
5	447 ^a	315 ^b	378 ^b	207 ^c	165 ^c	63	<0.04
6	310	231	215	202	152	43	NS
7	370 ^a	356 ^a	216 ^b	108 ^c	206 ^b	39	<0.002
8	311	169	166	251	159	57	NS
9	304 ^a	334 ^a	134 ^b	139 ^b	103 ^b	51	<0.02
10	356 ^a	331 ^a	143 ^b	176 ^b	158 ^b	46	<0.015
11	286 ^a	244 ^{ab}	218 ^b	135 ^c	154 ^c	30	<0.022
12	305 ^a	198 ^b	237 ^c	198 ^b	126 ^d	35	<0.043
13	386 ^a	370 ^a	131 ^b	138 ^b	148 ^b	60	<0.016
14	292 ^a	297 ^a	151 ^b	78 ^c	97 ^{bc}	54	<0.034
15	188	197	175	96	81	48	NS

BXB Scottish Blackface, SWXB Swaledale X Blackface, CHXB Cheviot X Blackface, LLXB Lleyn X Blackface and TXB Texel X Blackface. Means with different superscript indicate significant differences (P<0.05)

Conclusion The results of this trial showed Scottish Blackface ewes to have larger within breed spatial distribution than the other breeds on free ranging hill conditions. Swaledale X ewes showed similar spatial differences to the Blackface on many occasions. Lleyn and Texel X ewes, show higher preference to be closer to members of their own breed with smaller spatial differences, thus display seemingly stronger social bonds within breed. Better knowledge of breed grazing patterns, and spatial distribution could be an important tool in managing biodiversity on hill environments.

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Cluster analysis of farming systems according to predisposing factors of lameness in dairy sheep

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Introduction Work on lameness has been focused on meat sheep but there is limited information on dairy sheep. Lameness is a welfare problem, which reduces productivity and it is a major problem in most sheep keeping countries (Winter, 2008). The latter is important for Greece which is ranked second (after Italy) in milk sheep production in Europe (deRancourt et al. 2006). The objective of this study was twofold. Firstly, to characterise the farming system in a representative sample of dairy sheep flocks, and to categorise them in certain clusters in relation to predisposing factors of lameness. Secondly, to assess the prevalence and the major epidemiological characteristics of lameness.

Materials and methods Data from 67 commercial dairy flocks of the Chios Sheep Breeders Cooperative "Macedonia" were used. A questionnaire for in-depth interviews was devised comprising questions about animals, facilities, equipment, management practices, nutrition, and major health problems with emphasis on lameness. Following the completion of the questionnaire the next step was clustering of the flocks. For this reason a K-Means Cluster Analysis was performed using the SPSS K-Means procedure that identifies relatively homogeneous groups of cases based on selected characteristics. The cluster analysis employed three characteristics of the farms (Stocking density, Flock size, Pasture), which were considered to influence the prevalence of lameness. An initial number of eight clusters were set *ad hoc*. Three dominant clusters (2, 3 and 7) were identified; each one comprising 10 or more flocks. Three flocks from each dominant cluster were randomly selected. These flocks were visited every 14 days for one lactation period (January to July 2008). Lameness was marked at the milking parlor and then separated from the flock. A detailed clinical examination was undertaken together with body condition scoring, locomotion scoring (LS), and assessment of lesions in individual claws. Body condition scoring and locomotion scoring were repeated every 14 days until the sheep was sound.

Results Cluster analysis identified 8 clusters, as shown in Table 1. Table 2 illustrates the key foot problems in the 3 main clusters. There was a significant prevalence of lameness in flocks in cluster 3 (13.14%), whereas it was lower in flocks of clusters 7 and 2 (6.1% and 3.1% respectively). The severity of lameness was high (LS > 3) in 48.2% of the clinical cases, whereas about 10.1% of cases were classified as light lame (LS = 2). The incidence of lameness was highest in February and April. Most of the cases were associated with lesions at the hind limbs (60.2%; there were no differences between left and right foot. White line lesions (including white line abscess) and footrot were the most important reasons for lameness in clusters 7 (58.3%) and 3 (82.8%). In 32/110 cases the duration of lameness was over two weeks, whereas in 19/32 lameness lasted more than one month. Footrot and Pedal joint abscess have the greatest severity followed by white line lesions.

Table 1 The eight clusters

Cluster	Number of flocks	Pasture availability	Average flock size	Average stocking density (m ² /ewe)
1	8	No	62	2,79
2	12	Yes	259	2,16
3	10	No	174	1,76
4	4	No	483	2,06
5	6	Yes	319	2,37
6	3	Yes	413	1,98
7	22	No	113	2,23
8	2	No	600	2,04

Table 2 Foot problems in 3 main clusters and their distribution according to Locomotion score and lameness duration

Foot Condition	Cluster 3 (n=487)	Cluster 7 (n=361)	Cluster 2 (n=770)	Total	Locomotion Score				Lameness duration		
					2	3	4	5	(<14 days)	(14-28 days)	(>28 days)
Footrot	53	15	5	73	8	31	29	5	51	9	13
White line disease	8	3	2	13	1	5	7	0	11	1	1
White line abscess	3	3	12	18	2	8	7	1	13	1	4
Pedal Joint abscess	0	1	2	3	0	0	2	1	0	2	1
Injury	0	0	3	3	0	2	0	1	3	0	0
Total	64	22	24	110	11	46	45	8	78	13	19

Conclusion Results showed a considerable prevalence of lameness in intensively reared flocks of Chios Sheep. Knowledge of the predisposing factors for lameness will assist farmers and veterinarians in making a correct management plan. Further work is required to investigate the relationship between lameness and milk yield in dairy sheep.

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The effect on lamb vigour of adding supplementary vitamin E to ewe diets in late pregnancy

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Introduction Neonatal lamb mortality represents both a welfare issue and an important production inefficiency. Approximately 80% of lamb mortality can be attributed to the starvation-mismothering-exposure complex and occurs in the first 3 days after birth. Sub-optimal supply of trace elements and vitamins to the ewe is a potential risk factor in lamb mortality with Se, vitamin E and fatty acids the most likely candidates (Rooke et al. 2008). Responses to vitamin E supplementation above requirement in the last third of gestation are variable probably because of differences between studies in route of administration, dose administered and pre-experiment vitamin E status of the ewe population. The object of the experiment was to characterise responses in lamb viability to supplementation of the maternal diet with vitamin E above the stated requirement.

Materials and methods Twin-bearing cross-bred ewes (approximately 21 months of age) were mated with either Suffolk or Texel rams. At housing, ewes were blocked by weight and randomly allocated to treatment groups by weight to give 4 treatment groups of 20 ewes, 10 of which had been mated with Suffolk and 10 with Texel rams. The ewes were housed in straw-bedded pens (20 ewes per pen) which were randomly allocated to one of the 4 nutritional treatments. Ewes were fed hay *ad libitum* throughout. Experimental diets were introduced approximately 6 weeks before lambing. Ewes were fed concentrates to meet energy requirements for stage of pregnancy with an additional 100g soya-bean meal per head per day. Supplementary vitamin E supplied 50, 100, 150 or 250 IU per head per day. Blood samples were obtained prior to introduction of diets; 17 days after introduction of diets and within 24 h of lambing from a subset of 8 ewes per treatment (32 total). Colostrum samples were obtained from as many ewes as possible 12 h after birth of the first lamb. All births were observed and the times of the following behaviours recorded for each lamb: birth; shaking of head; on knees; attempting to stand; successfully standing; reaches udder; attempts to suckle; successfully suckles. From these data a lamb vigour score was calculated (5 min after birth) where 0 = lamb standing or has stood and 4 = lamb lying flat and unable to raise head. At 1 and 12h after birth rectal temperature and at 12 h after birth, sex, crown-rump length and weight of each lamb were recorded. Feed, colostrum and plasma samples were analysed for vitamin E concentration. Parametric data were analysed using a 2 (sire) x 4 (vitamin E concentration) design. Effects of vitamin E were assessed using orthogonal polynomials. Non-parametric data were analysed using the Kruskal Wallis test.

Results Measured supplementary vitamin E concentrations in feed were in line with those expected (r^2 0.94, $P < 0.001$, $n=12$). Mean ewe plasma α -tocopherol concentration prior to introduction of the diets was 1.5 $\mu\text{g/ml}$ (s.e. 0.09) and did not differ between groups. There were linear effects of dietary vitamin E (Table 1) on plasma α -tocopherol 17 days after introduction of diets but not after lambing ($n=8/\text{treatment}$). However colostrum α -tocopherol was increased by supplementary feeding ($n=10/\text{treatment}$). Lamb vigour scores were superior for lambs sired by Texel rather than Suffolk rams (1.71 v 2.09, $P < 0.001$) but there were no differences as a result of vitamin E supplementation (average $n=38/\text{treatment}$). Lamb mortality was low in the first week of life (5/158 lambs) and unrelated to either sire or supplementary vitamin E. Lamb birth and weaning weights were unaffected by vitamin E supplementation.

Table 1 α -tocopherol ($\mu\text{g/ml}$) concentrations in ewe plasma and colostrum and lamb vigour scores

Vitamin E (IU/ day)	50	100	150	250	s.e.d.	Significance
Plasma						
Day 17	1.3	1.2	1.4	2.1	0.20	$P < 0.001$
Post-lambing	1.9	1.3	1.1	1.5	0.34	NS
Colostrum	4.6	8.6	8.7	14.5	1.69	$P < 0.001$
Lamb vigour score	1.86	2.02	1.91	1.80		NS

Significance of linear effect of supplementary vitamin E; quadratic effects, NS.

Conclusion Despite supplementary vitamin E eliciting positive responses in ewe plasma and colostrum vitamin E status and the lamb scoring system being sensitive to differences in vigour arising from sire, supplementary vitamin E had no effect on any lamb measurements. Merrell (1999) reported positive responses at levels of supplementation similar to those used in the current study. Possible explanations for differences between studies are differences in base-line plasma vitamin E concentration (lower in Merrell, 1999), ewe genotype and forage fed.

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Prediction of parturition in dairy cattle using a 2D accelerometer to measure lying behaviour – a preliminary observational field study

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Introduction There is a trend in the UK towards larger dairy herds increasing the number of cows per stockperson and reducing available time for routine observations. A slightly difficult calving can have an economic loss of £110 with up to £400 for a difficult calving (McGuirk *et al*, 2007). These losses are incurred in lost milk production, increased labour costs and delayed calving to conception interval. Therefore, it would be advantageous for the stockpersons to have an automated alert of the onset of calving as soon as possible to allow assistance to be given if required and to highlight cows for observation.

Miedema *et al* (2008) and Huzzey *et al* (2005) indicated that the frequency of transitions between standing and lying changes in the hours preceding calving.

The aim of the study was to evaluate the potential use of automatic assessment of lying behaviour to predict the onset of calving in dairy cattle.

Materials and methods Six lactating dairy cows parity (mean $\pm$ SE) 2.4 ± 0.43 were recruited to the study 2 weeks before expected calving date. The time of calving was recorded. The cows were housed in a deep litter straw yard for 3 weeks prior to expected calving date; the cows were then moved to an individual straw pen with their calves for 2-3 days following parturition and to a cubicle yard with rubber mats and sawdust bedding following separation from the calf. Lactating cows were milked twice daily. Activity monitors (IceTagTM, Ice Robotics Ltd, Roslin, UK) were attached to the back right leg above the fetlock 2 weeks before expected calving date to measure standing and lying behaviour automatically. IceTags have been validated by Munksgaard *et al* (2006) for use on dairy cattle to record time spent standing and lying. 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. Frequencies of lying bouts were calculated for the 12 hours before and after calving. 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 90%. Frequency of lying was calculated as mean number of times a cow lay down in each 1 hour period.

Results

Figure 1 Lying bouts (n=6 cows) recorded from 12 hours before to 12 hours after calving (hour 0)

Figure 2 Activity of cows (n=6) recorded from 12 hours before to 12 hours after calving (hour 0)

There was a tendency ($p=0.06$) for the mean number of lying bouts to increase from -5 hours before calving (1.8 ± 0.31) to -3 hours pre calving (4.0 ± 1.37); before declining ($p=0.11$) to a trough at 1 hour after calving (1.2 ± 0.48). There was a similar increase in activity between 5 and 4 hours pre-calving. Activity levels increased ($p=0.08$) between -5 and -4 hours before calving (4.2 ± 0.5 min/hour and 2.0 ± 0.5 min/hour). Lying time is not presented in this abstract however lying time was higher before calving and then declined after calving. There was a large amount of variation surrounding the data which can be explained by the small number of animals on the study.

Conclusion The data presented suggest that it might be possible to alert staff to cows which may be expected to calve 4 hours before calving takes place. This may be particularly important on units with high cow to staff ratios. Further research is needed on a larger number of cows to confirm the potential to predict calving using automated recording of lying behaviour and activity.

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Changes in lying behaviour and activity during the peri-parturient period in primiparous and multiparous Holstein dairy cows

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Introduction The peri-parturient period is unsettling for dairy cows as they move from the dry period, through parturition, cow-calf separation and into the milking herd. The changes in physiological state, environment and social grouping through this period are likely to be particularly manifest in primiparous cows. Lactating dairy cows may be expected to spend up to 13.8 hours/day lying (Tucker and Weary, 2007), this has been shown to alter through the peri-parturient period (Huzzey *et al* (2005). The mean lying time for healthy cows at this farm was 11 hours day (Blackie *et al*, 2008). The aim of the present study was to assess the changes in activity levels of first parity cows before and after calving as compared to their multiparous herd mates.

Materials and Methods Seventeen lactating dairy cows parity 2.6 ± 0.24 (mean $\pm$ SE) were recruited to the study two weeks before expected calving date. The cows were housed in a deep litter straw yard for three weeks prior to expected calving date; the cows were then moved to an individual straw pen with their calf following parturition for two to three days. Cows were then moved to a cubicle yard (cow: cubicle ratio 1:1) with rubber mats and sawdust bedding. Cows were milked twice daily after calving. Activity monitors (IceTag[™], Ice Robotics Ltd, Roslin, UK) were attached to the back right leg above the fetlock two weeks before expected calving date 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. Mean time spent standing, lying and active was calculated from five days prior to calving to 10 days following calving. 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 90%. Frequency of lying bouts was calculated as mean number of times a cow lay down in a period of 24 hours. Differences between parity groups over time were calculated using repeated measures ANOVA and differences between different periods over the study were calculated using ANOVA and Tukeys post hoc test.

Results

Figure 3 Time spent lying (hours/day) in during the transition period, day 0 = day of calving (n=17) different superscripts indicate significant differences

Figure 4 Lying time (hours/day) from 5 days before to during the 10 days after calving in primiparous (n=3) and multiparous cows (n=14)

Cows showed a significant reduction ($p < 0.05$) in lying time between the day of calving (day 0) and days 1-3 following calving and similarly there was a significant difference in time spent lying down between the 3 days prior to calving and the 3 days after calving but not subsequent days. Cows spent approximately 12.5 hours lying down pre calving. Time spent lying down declined on the day of calving and the recovered to approximately 11 hours within a week of calving which is similar to mean values seen on this farm.

Conclusion Cows spent less time lying down during day's 1-3 post partum. No statistical difference was seen between multiparous and primiparous cows during the study period. However on days 2 to 5 following parturition mean lying time was 2.8 hours per day less in parity one cows in comparison to multiparous cows which may be biologically significant. The primiparous cows may not have had previous experience of cubicle housing which may negatively effect their lying when they are moved into the free stall yard. This study highlights the importance of management of first parity cows through the transition period and would benefit from being repeated with a larger number of animals.

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Is there a relationship between the behaviour of beef cattle and emissions of methane and ammonia from feedlot systems?

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Introduction Associations between animal behaviour and emissions of methane (CH₄) and ammonia (NH₃) have been noted in studies of grazing cattle (Lockyer, 1997) and feedlot confined cattle (Harper *et al.*, 1999, Flesch *et al.*, 2007). Methane emissions have been predicted as being greatest during bouts of rumination (Harper *et al.*, 1999) whereas the emissions of the indirect greenhouse gas ammonia tends to be low early in the morning but increasing rapidly in the early afternoon after which a rapid decline until sunset (Flesch *et al.*, 2007). With the exception of Harper *et al.*, (1999) there are few complete data sets that examine the interaction between animal behaviour and greenhouse gas emissions from intensive animal production systems. The current study aimed to investigate the relationship between animal behaviour and emissions of CH₄ and NH₃ in a beef feedlot system in northern Australia.

Materials and methods A typical beef feedlot system (Dalby, Queensland, 27.1°S, 151.2°E) was selected for measurements of methane and ammonia, and animal behaviour. Open path tuneable diode lasers (Boreal Laser Inc., Alberta Canada) and FTIR mass spectroscopy (University of Wollongong, NSW, Australia) were used to measure greenhouse gas concentrations. A three-dimensional sonic anemometer coupled with a high speed data logger (Campbell Scientific, Utah USA) located within the feedlot measured wind and turbulence statistics. WindTrax software (Thunder beach scientific, Canada) and the data collected for concentration of gases and micrometeorology were used to generate flux measurements using a backward Lagrangian stochastic model (bLS) (Flesch *et al.*, 2007). A single pen (161 cattle: 477 kg live weight: Brahman X) were selected for behavioural observations. The ration offered (11.75 kg DM/ day; 12.8 MJ/kg DM, crude protein 135 g/kg DM) comprised sorghum silage (0.15), straw (0.03), sorghum grain (0.55), cottonseed meal (0.22) and vegetable oil (0.05). The animals were fed once daily between 10.30 and 11am. Continuous time close circuit recording was used to observe behaviour between 6 am and 6 pm. Behaviour was categorised into feeding, ruminating, lying, standing, walking and other (Mitlohner *et al.*, 2001). A rumination index was calculated and regression used to assess the relationship between activity/ rumination index and production of CH₄ and NH₃ (Harper *et al.*, 1999).

Results

Average emissions of methane (■), N₂O and ammonia (◇) were 165.56 ± 87.34 g CH₄/head/day, 5.42 ± 7.78 g N₂O/head/day, and 114.85 ± 67.85 g NH₃/head/day. Ammonia emissions were similar to that observed by Flesch *et al.* (2007). Ammonia emissions were more strongly correlated with temperature (R² 0.53) than with animal activity index (R² 0.18), although there was a significant relationship with animal activity (P<0.05). Methane emissions were characterised by a small peak in the early morning (temperature 17.8°C), and increases in the late evening (temperature 24.7°C). Methane emission was significantly (P<0.05) correlated with rumination R² 0.23).

Conclusions

Ammonia emissions were more highly correlated with temperature than animal behaviour (a not unexpected relationship reflecting volatilisation rates from the surface of the feedlot). Methane emissions showed a significant correlation with rumination index; however the results suggest that much of the variation in emission was not related to behaviour and the increase in methane concentration in the late afternoon/evening reflected an artefact of microclimate conditions (3 pm temperature 33.1°C, 11 pm temperature 24.7°C).

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Effects of low protein diets on fat deposition in the whole body and within the muscle and subcutaneous adipose tissue of pigs

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Introduction Protein is an expensive nutrient in pig diets and high protein diets lead to high N excretion, a source of pollution. Low protein nutritional strategies are therefore needed, but these often increase fat deposition. This study compared three nutritional strategies differing in protein and energy supply using genotypes with different propensities for fat deposition.

Materials and methods One hundred and ninety-two entire male pigs; half 0.75 Duroc (D) and half 0.75 Large White (LW) were fed *ad libitum* from 40 to 120 kg live weight on one of three nutritional strategies (Table 1). The pigs were group fed, eight animals per pen, on a breed x nutritional strategy basis, over four replicates. The dietary protein content was reduced across the strategies, with the concentration of lysine and other amino acids being reduced in relation to protein. The pigs were reared at Harper Adams then transported to the University of Bristol abattoir, where they were slaughtered and dissected as described by Brown and Wood, (1979). Data were analysed using GLM with breed and nutritional strategy as factors.

Table 1 Nutritional strategies

Wt range	Baseline (B)			SOTA ^d			L P ^e		
	DE ^a	CP ^b	L ^c	DE	CP	L	DE	CP	L
40-65	13.5	210	12	14.0	195	12	14.0	195	12
65-90	13.5	210	12	13.5	180	11	13.5	165	10
90-120	13.5	210	12	13.0	170	10	13.0	130	7

^a MJ Digestible Energy/kg ; ^b g/kg crude protein; ^c g/kg lysine; ^d 'state of the art'; ^e Low protein

Results Table 2 shows several significant breed x nutritional strategy interactions for different measures of fat within the empty body (carcass plus red and green offal components). The values in Duroc were similar across the nutritional strategies, rising slightly in some cases from B to SOTA to LP, while a greater response to nutritional strategy was found in LW, where the amount of fat increased significantly across the strategies in the order Baseline < SOTA < Low N. The same pattern was also found for the concentrations of intramuscular fatty acids in the *longissimus dorsi* muscle (LD) and in the subcutaneous adipose tissue depot in LW.

Table 2 The effects of breed x nutritional strategy on overall fat distribution

Variable	Duroc			LW			S.E.D.	P value
	B	SOTA	L P	B	SOTA	L P		
<u>Body measurements</u>								
Empty body weight (kg)	105.4	104.7	105.8	107.5	106.1	107.4	1.40	>0.05
P2 fat depth (mm)	17.9 ^b	18.9 ^b	19.0 ^b	13.8 ^a	15.0 ^a	18.4 ^b	0.93	0.013
Fat % EBW	15.5 ^b	15.7 ^b	15.9 ^b	12.1 ^a	13.7 ^a	16.9 ^b	0.77	<0.001
<u>EB fat depots (kg)</u>								
Total fat	16.5 ^{bc}	16.5 ^{bc}	16.8 ^{bc}	13.0 ^a	14.5 ^{ab}	18.2 ^c	0.88	<0.001
Subcutaneous	11.9 ^b	11.8 ^b	11.9 ^b	9.8 ^a	10.1 ^{ab}	13.4 ^b	0.59	<0.001
Intermuscular	3.5 ^b	3.7 ^b	3.7 ^b	2.6 ^a	3.0 ^{ab}	3.8 ^b	0.28	0.018
Thoracic (g)	44.3 ^a	51.1 ^{ab}	51.5 ^{ab}	41.9 ^a	45.2 ^a	66.0 ^b	6.00	0.035
Flare (g)	809.1 ^{ab}	851.3 ^b	838.8 ^b	566.0 ^a	699.3 ^{ab}	1007.3 ^b	0.07	<0.001
Omental & mesenteric (g)	151.3	149.3	162.5	124.1	130.7	156.0	16.80	>0.05
<u>Total fatty acid weights</u>								
LD muscle (mg/100g)	1785	1946	2078	914	957	1185	161	>0.05
Subcut adipose (g/100g)	73.9 ^{ab}	74.1 ^{ab}	73.9 ^{ab}	70.8 ^a	71.7 ^a	75.5 ^b	1.30	0.018

SED standard error of difference, maximum quoted. Means in a row with the same superscript do not differ significantly. Tukey-Kramer test at the 0.05 level, *post-hoc*.

Conclusions These results show that different pig breeds respond differently in terms of fat deposition to the levels of nutrients supplied, particularly protein and energy. Therefore it is necessary to balance diets to genotypes, especially for leaner genotypes such as LW, to avoid excess levels of fat deposition within the body.

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Effect of pig growth rate and health status on meat eating quality

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Introduction Growth rate is an important performance indicator in pig production and therefore influences profitability. There is also evidence that faster growing pigs have superior tenderness (MLC, 1989), possibly linked to faster muscle protein deposition through the activity and expression of proteolytic enzymes. Slow growth could also result in higher boar taint levels in entire male pigs because they will be older and sexually more mature at slaughter (MLC, 1989). The current project was therefore undertaken to investigate the effect of variations in growth rate on tenderness and boar taint.

Materials and methods Two hundred and twenty five loins were examined from Large White x Landrace pigs involved in a 3 x 2 x 2 factorial experiment. Factors were 3 growth categories (Fast, Slow and Interrupted), 2 sexes and two final live weights, 90 and 110kg. Growth rate was measured between weaning and slaughter, with pigs fed *ad libitum*. Guidelines for fast growth were >600 g/d to 90kg and >700 g/d to 110kg. Guidelines for slow growth were around 500 g/d to 90kg and 590 g/d to 110kg. Interrupted growth pigs were those that had suffered a period of weight loss presumed to be due to infection. Final numbers in the 3 growth categories were 96 fast, 86 slow and 43 interrupted. Loins were delivered to Bristol University where various meat quality measurements were made. The loins were conditioned at 1°C for 10 days then stored at -20°C before analysis. A Stable Microsystems Texture Analyser fitted with Volodkevitch jaws was used to measure toughness. Skatole and androstenone concentrations in subcutaneous fat from the loin region were measured using the procedures of Whittington *et al.* (2004). Steaks 2.5cm thick were presented to a 10 member taste panel for sensory analysis after griddling to 72°C internal temperature. Data were statistically analysed using general linear models (GLM), with growth category, carcass weight group and sex as factors and including interaction terms.

Results The growth rate, fat thickness, toughness and skatole measurements from both sexes and carcass weights are presented in Table 1. P₂ fat thickness was approximately 1mm greater in the Fast than the Slow and Interrupted groups. There was a strong tendency for toughness to be lower in the Fast group but a significant growth category x carcass weight interaction prevented this being a statistically significant effect. Results in Table 2 show that toughness was significantly lower in the Fast groups at both 90 and 110 kg carcass weight.

Table 1 Growth rate, P₂ fat thickness, toughness and skatole in growth category groups

Variable	Growth category			p-value	sig.	sed
	Fast	Slow	Interr			
Finisher ADG (kg)	0.74 ^a	0.57 ^b	0.56 ^b	†	*	0.036
P ₂ (mm)	10.13 ^a	9.06 ^b	8.43 ^b	<0.001	***	0.351
Toughness (kg)	4.40	5.22	5.41	*	ns	0.405
Skatole (µg/g)	0.068 ^a	0.052 ^b	0.054 ^{ab}	0.005	**	0.007

† * Sig. Interactions between sex and growth category and carcass wt and growth category respectively

Table 2 Effects of growth category on toughness in carcass weight groups

Weight	Growth category Toughness (kg)			p-value	sig.	sed
	Fast	Slow	Interr			
90 kg	4.49 ^b	4.81 ^{ab}	5.35 ^a	0.019	*	0.305
110 kg	4.32 ^b	5.63 ^a	5.47 ^a	<0.001	***	0.272

The results for skatole and androstenone in males are in Table 3. The concentrations of both the compounds were highest in the Fast group. There were no significant differences in taint compound concentrations between 90 and 110kg carcass weight groups in males. The results for eating quality for Fast and Slow groups only for males weighing 110kg are in Table 4. Tenderness was higher in the fast group; abnormal odour and flavour were not significantly different between the groups.

Table 3 Effects of growth category on skatole and androstenone concentrations in males (µg/g)

Variable	Growth category			vr	p-value	sig.	sed
	Fast	Slow	Interr				
Androstenone	0.500	0.383	0.397	3.21	0.045	*	0.062
Skatole	0.080 ^a	0.056 ^b	0.060 ^{ab}	4.90	0.009	**	0.011

Conclusions The results show that pork tenderness was increased in pigs growing relatively rapidly in the finishing period. Skatole and androstenone concentrations were not higher in slow growing males and odour/flavour scores were not reduced. Therefore fast growth will benefit tenderness but not necessarily odour/flavour.

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Table 4 Eating quality of griddled loins steaks (1 to 8 scales). Comparison of Fast and Slow groups in 110 kg males

	Growth category				
	Fast	Slow	p-value	sig.	sed
Pork odour of fat	3.73	4.20	0.025	*	0.205
Abnormal odour of fat	2.69	2.50	0.438	ns	0.241
Tenderness	4.22	3.81	0.004	**	0.139
Pork Flavour	3.95	3.91	0.804	ns	0.189
Abnormal Flavour	2.86	3.05	0.390	ns	0.217
Flavour liking	4.50	4.20	0.107	ns	0.183
Overall liking	4.33	3.91	0.020	*	0.179

The use of glycerol in diets for finishing pigs

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Introduction The production of fuel from renewable energy sources has generated increasing interest in recent years and biofuel production is being encouraged within the United Kingdom. If current government targets are to be met, approximately 2.7 million tonnes of oil seed rape are needed to supply biodiesel which will result in a significant amount of the by-product glycerol. Glycerol may be a useful source of energy in diets for pigs but there is limited research on the optimum level of inclusion, digestible energy (DE) content or on the effect on performance. Research by Mourot *et al* (1994) suggested that glycerol tended to reduce performance but significantly improved meat quality as assessed by drip loss. The aim of this study was to investigate the effect of glycerol inclusion in finishing pig diets on performance and meat quality.

Materials and methods Four experimental diets were produced to contain 0, 40, 80 or 120g/kg glycerol, 185g/kg crude protein, 9.7g/kg lysine and 14.2MJ/kg DE (fresh basis). Glycerol replaced wheat in the diet formulation. The basal diet contained (g/kg) wheat 437, barley 300, soyabean meal 227, vegetable oil 13.6, limestone 9, dicalcium phosphate 3.5, salt 4.1, lysine 0.8 and minerals and vitamins 5. A performance trial was conducted utilising 48 pigs housed in groups of six from 14 weeks of age until slaughter at either 152 or 159 days. Feed was supplied *ad libitum* through electronic ACEMA feeders and individual pig intake, intake per feeder visit, time of visit, duration of visit and number of visits were recorded on a daily basis. Pigs were slaughtered under commercial conditions, coldweight and backfat at the P₂ position recorded and killout percentage (KO%) calculated. A 2-inch chop was taken for meat quality analyses (cooking loss, drip loss, sarcomere length and shear force) (Beattie *et al* 1999).

Results There was no significant effect of glycerol inclusion on feed intake or FCR although average daily gain decreased when glycerol was included at 40 or 120g/kg (Table 1). Feeding behaviour was significantly affected by glycerol inclusion with the number of visits in a 24hr period increasing linearly ($P < 0.001$) with increasing glycerol content. However, the total time spent at the feeder (per pig/24hr) decreased linearly ($P < 0.01$) as glycerol inclusion increased. There was no significant effect of glycerol inclusion on carcass or meat quality assessments.

Table 1 Effect of glycerol on performance (14-21wks with 14wk weight as co-variate), feeding behaviour and meat quality

	0g/kg	40g/kg	80g/kg	120g/kg	SEM	P	P=Lin
Feed intake (g/d)	2527	2286	2528	2395	119.7	NS	NS
Average daily gain (g/d)	1031 ^b	927 ^a	995 ^{ab}	905 ^a	33.9	<0.05	NS
Feed conversion ratio	2.45	2.47	2.55	2.66	0.103	NS	NS
Total number of feeder visits (pigs/24hr)	4.9 ^a	16.0 ^b	14.3 ^b	20.9 ^b	2.80	<0.01	<0.001
Total time at feeder (pig/24hr)	2.82 ^b	1.39 ^a	1.89 ^a	1.34 ^a	0.260	<0.01	<0.01
Kill out (%)	74.6	76.8	76.3	76.4	0.79	NS	NS
Backfat at P ₂ (mm)	13.8	14.0	13.6	13.6	1.16	NS	NS
Cooking loss (%)	28.8	30.3	28.8	29.1	1.19	NS	NS
Drip loss (%)	4.7	3.8	4.9	4.6	0.64	NS	NS
Sarcomere length (mm)	2.0	1.9	1.9	1.8	0.05	NS	NS
Shear force (kg/cm ²)	3.6	4.1	3.8	3.7	0.19	NS	NS

^{a,b} Treatment means with different superscripts are significantly different.

Conclusions Glycerol inclusion significantly affected ADG but the effect was not linear. Feed intake and FCR were not significantly affected by glycerol inclusion but there was a tendency for FCR to become less efficient as glycerol inclusion increased. These findings are in keeping with Mourot *et al* (1994) and suggest that the digestible energy (DE) content of glycerol is lower than for wheat although more work is required to establish the DE content of glycerol. While there was no significant effect of glycerol inclusion on daily feed intake, variability between treatments was high. Glycerol inclusion increased the number of visits to the feeder but decreased the total time spent at the feeder which may be due to reduced palatability (Brief and Davis 1982). The lack of effect on carcass or meat quality parameters is in contrast to the results reported by Mourot *et al* (1994).

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The effect of dietary regime and method of grouping on the variation in growth rate between pigs

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Introduction A high degree of variable growth rate between pigs of the same age has been associated with overall poor herd performance and decreased profitability (Magowan *et al.*, 2007). The aim of the current study was to reduce the variation in growth rate between pigs by grouping them in either uniform or mixed weight groups at weaning and offering them either 'special' or 'normal' dietary regimes during the post weaning and finishing periods.

Materials and methods Over six time replicates, 960 pigs (Landrace x Large White) were randomly allocated into groups of 20 at weaning (28 +/- 2 days of age) according to weight and sex. For each replicate, eight groups were formed. Six groups contained pigs of uniform weight i.e. two groups were comprised of all 'light' weight pigs (average weight 7.1kg), two groups all 'medium' weight pigs (average weight 8.9kg) and two groups all 'heavy' weight pigs (average weight 10.4kg). The remaining two groups contained a mixture of heavy, medium and light weight pigs i.e. mixed weight groups (average weight 8.8). During the post weaning period pigs were offered either a high allowance of starter diets (4kg/pig of starter 1 diet followed by 8kg/pig of starter 2 diet) ('High') or a low allowance (2kg/pig of starter 1 diet followed by 4kg/pig of starter 2 diet) ('Low'). A standard grower diet was offered to 11 weeks of age. Starter diet allowance was balanced across groups. When pigs were transferred to finishing accommodation (10 weeks of age) each group of 20 pigs was split into 2 similar groups of 10. At 11 weeks of age groups were randomly allocated either a 'Special' (DE 14.5MJ/kg, total lysine 1.1g/kg) (S) or 'Normal' (DE 13.5MJ/kg, total lysine 0.95g/kg) (N) finishing diet so that 'finisher' diet was balanced across groups and previous post weaning dietary regime. Overall there were four dietary regimes from wean to finish ('Low + N', 'Low + S', 'High + N' and 'High + S') and pigs were grouped in either uniform or mixed weight groups. Pigs were weighed and the coefficient of variation (CV) for weight and average daily gain (ADG) was calculated at weaning, 7, 10, 11, 15 and 20 weeks of age. Results were analysed by Analysis of Variance (ANOVA) using Genstat (6th Edition).

Results There were no significant interactions between dietary regime and method of grouping. Dietary regime had no significant effect ($P>0.05$) on the CV for weight of pigs (wean, 10 and 20 weeks of age: 0.103, 0.140 and 0.126 respectively) or on the CV for ADG from weaning to 10 weeks of age (0.726, $P>0.05$). However, when pigs were offered 'High + N', the CV for ADG (11 weeks of age to finish) was significantly higher ($P<0.01$, SEM 0.0078) than when they were offered 'Low + N' and 'High + S' with 'Low + S' being intermediate (Figure 1). When the pigs in the light, medium and heavy groups were considered as one large group, the CV for weight when pigs were 11 and 15 weeks of age was significantly ($P<0.05$) lower in uniform weight groups than in mixed weight groups (Table 1). When the data for the light, medium and heavy weight groups were considered separately, the CV for wean, 10 and 20 week weight was significantly lower (0.077, $P<0.001$; 0.131, $P<0.001$ and 0.120, $P<0.05$ respectively) than that of the mixed weight groups (0.182, 0.168 and 0.144 respectively). It was noted that between wean and 10 weeks of age the variable weight of pigs in uniform groups almost doubled, whereas that of mixed weight groups decreased slightly. Between 10 and 20 weeks of age, the CV for both the uniform and mixed weight groups decreased by a similar proportion.

Table 1 Effect of method of grouping on the CV for weight of pigs (Uniform groups considered as one group)

	Mixed Groups	Uniform groups	SEM	P
Age:				
Wean	0.181	0.174	0.0042	NS
10 weeks	0.167	0.154	0.0064	NS
11 weeks	0.158	0.139	0.0056	<0.05
15 weeks	0.160	0.140	0.0065	<0.05
20 weeks	0.143	0.127	0.0067	NS

Figure 1 Effect of dietary regime on the CV for average daily gain of pigs between 11 weeks of age and finish.

Conclusion Uniform grouping of pigs at weaning appears to lower the variation in weight between pigs at 11 and 15 weeks of age and numerically at 20 weeks of age compared with pigs in mixed weight groups from weaning. Offering pigs a similar 'level of nutrition' i.e. 'Low + N' or 'High + S' through the post weaning and finishing periods appears to reduce the variation in growth rate between pigs during finishing compared with when the dietary regime includes a mixture of 'levels of nutrition' i.e. 'High + N' and 'Low + S'. If variation in the weight of pigs within a group is low when the group is formed e.g. at weaning, it will increase, whereas if it is high when the group is formed it will not.

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Evaluation of alternative methods to predict the lean meat percentage of pig carcasses

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Introduction The value of a pig carcass is largely based on its lean meat percentage (LM%). The ultimate method of accurately measuring the lean meat percentage is a full dissection of the carcass. However, this is both time consuming and expensive. Lean meat percentage prediction equations have been established for various ‘probes’ and indicator cuts. Although these prediction equations have low (less than 2.5) Root Mean Square Errors of Prediction (RMSEP) and high R^2 values, research continues to investigate other methods to produce equations with greater accuracy and to identify alternative techniques to full dissection. The aim of this experiment was to investigate the accuracy of lean meat percentage prediction equations using data gathered from 1) grading probes, 2) primal cuts and 3) photographs from cross sections of the loin.

Materials and methods A total of 161 pigs (Landrace x Large White) were selected according to sex, slaughter weight and back fat depth 65mm from the midline at the level of the last rib (P_2). The cold carcass weight of pigs ranged from 52.5 to 138.6kg and averaged 82.4kg, with a standard deviation of 13.5kg. Back fat depth at P_2 ranged from 5 to 25mm and averaged 12.0mm with a standard deviation of 3.5mm. After slaughter the back fat depth at P_2 and P_3 (65mm from the midline between ribs 9 and 10) was measured using the Ulster, Hennessy and Optical grading probes on the hot carcass. The right side of each carcass was dissected according to the method of Walstra and Merkus (1996). The loin, leg, shoulder and belly were fully dissected into muscle, subcutaneous fat, intramuscular fat and bone. Before dissection, the loin was cut into 4 sections. The loin was cut between the 9th and 10th rib, just after the last rib (P_2) and again a further 10cm towards the rump end of the loin. This 10cm segment was termed ‘Loin C’. These cuts exposed 5 cross sectional areas (A (shoulder end) to E (rump end)). Photographs of the 5 cross sections were taken at a fixed distance and magnitude. Using SigmaScan Pro, the muscle depth, width and area, subcutaneous fat area, intramuscular fat area and bone area were measured and the percentage lean meat of the cross sectional photograph calculated. The subcutaneous and intramuscular fat depth under the skin was also measured, perpendicular to the skin surface, at 4 equally spaced points along the circumference of the cross section. Point one was at the midline, and point 4 was above the lower end of the eye muscle with points 2 and 3 being intermediate. Regression analysis using Genstat (Version 11) was used to statistically analyse the results. The data set was randomly split with 2/3 of the data being used to establish the equation and the remaining 1/3 to validate the equation. An accurate equation was identified as one with having a high R^2 value (>0.65) and a low RMSEP value (≤ 2.5).

Results LM% prediction equations for the whole carcass with high R^2 and low RMSEP values were attained using the LM% values of the primal cuts i.e. loin, shoulder, leg and belly (Table 1). Using the LM % of ‘Loin C’, an accurate equation was also attained to predict the LM% of the carcass. Prediction equations using the Ulster, Hennessy and Optical grading probes had lower R^2 and higher RMSEP values compared with when the primal cuts and ‘Loin C’ were used but, values were still acceptable for use within the EU classification system. When P_3 was included as a variable within the equation for the Ulster, Hennessy and Optical probes, the R^2 and RMSEP did not improve significantly, compared with when only the P_2 value was used. The accuracy of the prediction equations using the LM% of the cross sectional areas measured from the photographs was comparable to equations produced using probe data. A stepwise regression using the data from the 5 photographs of each loin sample produced a prediction equation with a R^2 of 0.87 and a RMSEP of 1.43. The equation incorporated a total of 8 variables: the intramuscular fat area and depth of subcutaneous fat at point 2 on photo A, the depth of subcutaneous fat at point 4 on photo B, the eye muscle area, subcutaneous fat area and depth of subcutaneous fat at point 2 on photo C, the intramuscular fat area on photo D and the depth of subcutaneous fat at point 3 on photo E.

Table 1 The accuracy of prediction equations for lean meat percentage of the whole carcass using grading probes, primal and indicator cuts and photographic measurements

Predictor:	R^2	RMSEP	Predictor:	R^2	RMSEP	Predictor:	R^2	RMSEP
Loin LM%	0.82	1.72	Chop A LM% (photo)	0.67	2.25	Ulster Probe P_2	0.62	2.32
Shoulder LM%	0.87	1.31	Chop B LM% (photo)	0.65	2.33	Hennessy P_2	0.59	2.42
Leg LM %	0.89	1.39	Chop C LM% (photo)	0.60	2.56	Optical P_2	0.62	2.49
Belly LM%	0.80	1.73	Chop D LM% (photo)	0.54	2.36			
‘Loin C’ LM%	0.70	2.29	Chop E LM% (photo)	0.53	2.85			

Conclusion The use of grading probes to measure the back fat depth at P_2 and ultimately predict the lean meat percentage of a pig carcass produces acceptable levels of accuracy. However, a more accurate prediction can be made if the LM% of ‘Loin C’, the loin, shoulder, belly or shoulder is established. An accurate prediction of carcass LM% can also be made using a combination of various measurements e.g. fat depth and eye muscle area from cross sectional images of the loin.

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Using meta-analysis to determine the influence of dietary factors on pork quality

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Introduction Consumption of pork is declining in the UK, while demands of costumers for improved pork quality are rising. Various experimental studies suggest that dietary supplements such as vitamins and minerals could help to improve pork quality without negatively affecting other aspects of the production cycle (e.g. growth, welfare). However, results from different experiments are inconsistent, suggesting different magnitudes of effects caused by the same dietary supplementation. Meta-analysis (MA) is a statistical tool designed to combine results from different studies in a statistically sound manner to produce robust quantitative estimates of the impact of specific factors on traits of interest. In this study we tested the feasibility of meta-analysis for determining how supplementation of vitamin E as antioxidant reduces lipid oxidation and improves redness of pork over time, extending thus its shelf-life.

Materials and methods As outlined by Sauviant *et al.* (2008) and illustrated in Figure 1, meta-analysis consists of many iterative steps, starting from a careful definition of the study objectives, followed by systematic gathering, storing and statistically analysing of published data, derived from different experiments and the validation of the developed statistical meta-models. In this study the meta-analysis procedures were applied to investigate the effect of different vitamin E levels on lipid oxidation, measured as mg of malonaldehyde per kg meat thiobarbituric acid reacting substances (TBARS) over time. Based on the initial literature study 30 publications were selected, of which only six seemed appropriate for statistical analysis. The statistical analysis focused on studies in which animals were administrated with various doses of supplementary vitamin E all throughout their growth period. For data entry an SQL database was used and all known sources of variations like breed, dietary protein-, amino acid- and energy levels, rearing conditions, etc. were registered.

Graphical- and statistical analysis were carried out with SAS System for Windows Release 9.1 statistical software package (proc mixed, proc nlmixed). Various statistical models were developed to describe the effect of vitamin E on colour and lipid oxidation at particular times *post mortem* as well as their time trends, accounting for both within and between study variations. For post-analysis evaluation and model validation we used data sets which were not taken into account as primary source. The final users of the developed models will be scientists or industry, who will use the models to test hypothesis and to compare the model results to their own data.

Figure 1 Main steps of meta-analysis (Sauviant *et al.*, 2008)

Results Results for treatment effects of vitamin E over time are shown in Table 1. Supplement of vitamin E has an overall statistically significant effect ($p < 0.05$) on TBARS at different times post slaughter as long as vitamin E dose is over 100 IU/kg fed diet, however no significant differences have been detected between doses over 100 IU/kg fed diet. Our graphical- and statistical analyses suggest non-linear time trends for TBARS with a plateau reached at about 4 days post slaughter.

Table 1 LSMeans (SE) of TBARS for different treatments

VitE (IU)/Day	0	1-2	3-4	>4
0	0.13(0.12) ^a	0.39(0.11) ^a	0.50(0.12) ^a	0.69(0.10) ^a
100	0.20(0.14) ^a	0.25(0.11) ^{a,b}	0.30(0.13) ^b	0.41(0.10) ^b
200	0.09(0.12) ^a	0.16(0.11) ^b	0.11(0.16) ^b	0.26(0.10) ^b
700	0.13(0.06) ^a	0.14(0.07) ^b	0.20(0.09) ^b	0.10(0.07) ^b

a, b: Same superscripts refers to no significant differences ($p < 0.05$)

Conclusion Meta-analysis appears to be a feasible tool to examine how vitamin E affects TBARS, based on previous results from various experiments. The improvement of the power of meta-models requires additional data sources.

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The use of near infrared reflectance spectroscopy (NIRS) to predict the nutritive value of standard commercial diets for post-weaned pigs

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Introduction In order to assess the nutritive value of pig diets, performance and digestibility trials must be conducted as there is no accurate alternative to predict nutritive value. However, the use of near infrared reflectance spectroscopy (NIRS) to predict performance from feed ingredients has been shown to have potential. Owens *et al* (2007) investigated the use of NIRS to predict the performance of broilers offered wheat-based diets, through scanning of whole wheat, and observed that NIRS accurately predicted liveweight gain and gain:feed. The aim of this study was to investigate if NIRS could be used to predict the performance of pigs, through scanning of the complete diet.

Materials and methods Postweaning diets (n=173), formulated and produced by Devenish Nutrition Ltd, were offered to post weaned pigs in a series of performance trials. Pigs were weaned between 21 and 35d and offered Starter 1 diet for 12-14d postweaning and then Starter 2 diet until 24-28d post weaning (depending on specific experimental protocol). Pigs were weighed at weaning, at the end of Starter 1 allocation and at the end of Starter 2 allocation to calculate mean liveweight gain. Feed intakes were recorded on a pen basis and feed conversion ratio (FCR) was determined for each diet. There were six pen replicates per dietary treatment for all trials resulting in a total of 858 pens. Diets were milled (0.75 mm screen), dried for 24h (100°C) and scanned in duplicate on a Foss NIRSystem 6500 spectrophotometer at 2nm intervals over the visible and near infrared wavelength range. The optical data were recorded as log 1/Reflectance (log 1/R) values using the ISI-NIRS3 Version 4.00 software. Sub-sampling error was reduced by setting the root mean square (RMS) difference from the mean of each sub-sample at each wavelength to 700 μ log optical density (OD). Any spectra with RMS values above this limit were removed and the sample rescanned before mathematical transformations were performed. The spectra were trimmed to 1100 to 2498nm.

Results NIRS calibrations with high R^2 and low SEC (standard errors of calibration) were obtained for intake, liveweight gain and FCR. However, upon cross validation the predictions weakened with lower I-VR (R^2 of validation) values and higher SECV (standard error of cross validation) (Table 1). True validation was performed by removing every fifth sample from the dataset and producing calibrations based on the remaining samples. The independent samples were predicted and R^2 and standard error of prediction (SEP) calculated against the actual data. Only the prediction of gain for pigs offered Starter 2 diets resulted in reasonably robust R^2 and SEP values ($R^2 = 0.820$ and SEP = 0.44). However, the SEP expressed as % of the mean (7.54%) was unacceptably high.

Table 1 NIRS calibration and cross validation statistics for prediction of performance

Calibration and cross validation statistics							
	N	Mean	R^2	SEC	I-VR	SECV	SECV as % of mean
Starter 1							
Intake (kg)	164	3.08	0.813	0.215	0.707	0.269	8.75
Gain (kg)	166	2.80	0.810	0.248	0.666	0.328	11.73
FCR	161	1.11	0.787	0.052	0.664	0.065	5.92
Starter 2							
Intake (kg)	135	7.56	0.864	0.312	0.752	0.421	5.57
Gain (kg)	141	5.94	0.884	0.303	0.805	0.392	6.60
FCR	134	1.25	0.930	0.038	0.876	0.050	3.99

Conclusions While calibration and cross validation NIRS predictions resulted in high R^2 and low standard errors for the performance parameters, the independent validation proved the predictive equations were not robust. NIRS has been reported to accurately predict nutritive value; however, the majority of the studies in the literature do not independently validate the predictions. The strength of the current study is that the prediction equations were validated using an independent set of diets (n = 28) of the same type used to establish the equations. No strong relationships were apparent and it can be concluded that NIRS did not accurately predict performance of pigs offered standard commercial diets.

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Effect of a compensatory growth feeding regime on muscle glycogen and meat quality attributes in pigs

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Introduction Compensatory growth studies in pigs have indicated that their meat tends to have increased proteolysis and a faster rate of tenderisation (Kristensen *et al.*, 2002). The postmortem rate of muscle glycogen decline influences meat quality, for example in PSE. There are two forms of muscle glycogen, acid soluble macroglycogen (MG) and acid insoluble proglycogen (PG), which have different turnover rates *in vivo* (Graham *et al.*, 2001). The aim of this study was to determine whether a compensatory growth feeding regime would influence meat quality characteristics and whether the changes in feed availability during the trial would differentially alter the postmortem degradation of the different forms of glycogen.

Materials and methods Entire female pigs (25 kg, n=48) from day 60 of age were individually penned and *ad libitum* (A) fed a grower diet (13.9 MJ metabolizable energy and 165 g crude protein per kg diet), then subsequently divided into groups (n=8) and fed as indicated in Table 1. *Post mortem* pH was measured at 45 min (pH₄₅) and 24 h (pH_{24h}). Longissimus dorsi (20 g, LD) was taken at 0 and 48 h, snap-frozen in liquid nitrogen and stored at -80°C until glycogen analysis (Adamo and Graham, 1998). Dissected LD chops were conditioned at 4°C for 8 days, then frozen at -80°C until analysed for shear force (SF). Data were analyzed using the general procedure of Genstat (VSN International Ltd, UK). The model included main plot (slaughter group (SLG); slaughter at age day 114 (SLG1), 116 (SLG 2) and 156 (SLG3)) and sub plot (dietary treatment; A and restricted (R) feeding at 0.70 of A intake) within 8 blocks. Means were calculated and significance was when P<0.05.

Table 1 Feeding regimes of treatments

Group	SLG1		SLG2		SLG3	
	A	R	AL	R	A	R
60-73 day	Adjusted to A					
74-113 day	A	R	A	R	A	R
114-116 day			A	A	A	A
117-156 day					A	A

Results Irrespective of treatment, muscle MG was higher than PG (P<0.01; 87.9 and 26.0 mg/g respectively) at 0 h. When the decline of MG and PG over the first 48 hour postmortem was examined (MGD and PGD respectively) only MGD showed any significant effects. There was a trend for MGD to be less in SLG3 whilst for R groups it was higher. Overall there was a trend for an interaction between age and feeding regime with R feeding in SLG1 and SLG2 having a higher rate of MG depletion than A with this relationship being in SLG3. The SLG3 had the lowest SF value whilst R feeding gave a trend for tougher meat than A. At SLG2 the carcasses showed the lowest pH₄₅ whereas SLG3 showed lowest pH_{24h}. R feeding showed lower pH_{24h} than A.

Table 2 The effects of diet manipulation on the rate of glycogen depletion and meat quality attributes.

	SLG1		SLG2		SLG3		SED	Effects (P)		
	A	R	A	R	A	R		SLG	Feeding	Interaction
MGD ¹	35	46	50	65	36	33	10.2	0.063	0.033	0.085
PGD ¹	20	25	21	21	21	21	2.4	0.737	0.248	0.174
SF ²	6.3	7.6	6.5	7.7	5.5	5.6	0.73	0.015	0.062	0.491
pH 45 min	6.26	6.25	6.16	6.20	6.29	6.24	0.048	0.048	0.800	0.499
pH 24h	5.56	5.50	5.55	5.48	5.49	5.46	0.025	0.045	<0.001	0.323

¹ units; mg/gmuscle/48hr; ² units; kg

Conclusion In the trial SLG1 was included to examine the effects of restricting feed in taken between A and R groups whilst SLG2 examined the immediate effects of re-alimentation after the R period. Overall the compensatory growth feeding regime increased the rate of decline of MG in pigs subjected to R feeding but there was no affect on PG, suggesting that the feeding regime differentially alters the susceptibility of the two muscle glycogens. Although the dietary regime appears not to alter the rate of pH decline, as indicated by pH₄₅, it was associated with a lower ultimate pH, as indicated by pH_{24h}, and a higher SF. However, at the end of the compensatory growth period (SLG3) there was no significant difference in these meat quality attributes between the feeding regime groups.

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The effect of a mixture of encapsulated fatty acids on performance of growing pigs

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Introduction Efficiency of feed is of primary commercial importance in pig production. Interest in nutritional solutions to increase feed efficiency has increased since the bans/restrictions on the use of antibiotic feed additives and heavy metals. DeviGuard[®] is a mixture of short, medium and long chain fatty acids all of which are encapsulated to reach the hind gut. Fatty acids have been shown to be the preferred energy source of the enterocytes in the gut, active against pathogenic bacteria and anti-inflammatory. The aim of the present study was to assess the effects of fatty acid supplementation on the performance and digestibility of growing pigs.

Materials and methods In Treatment 1, control treatment, 20 pigs were offered a standard cereal soya diet (DE 14.8 MJ/kg, CP 190g/kg, lysine 12.5 g/kg) from 7 to 14 weeks of age. In Treatment 2, DeviGuard[®] treatment, 20 pigs were offered the same diet as control supplemented with 3 kg of DeviGuard[®]/tonne of finished feed hence pigs were offered approximately 3.5g DeviGuard[®] per day. Specification of the treatment diet was the same as the control diet (DE 14.8 MJ/kg, CP 190g/kg, lysine 12.5 g/kg). Pigs were housed individually and weights and feed intake were measured each week during the experimental period which started at 7 weeks of age and ended at 14 weeks of age. During the week when pigs were ten weeks of age, hence they had been on the experimental diets for 3 weeks, total faecal collections were carried out twice a day from the solid floor of the pen (no marker was used). The faeces was analysed for dry matter, crude protein and energy.

Results The inclusion of DeviGuard[®] at 3g/kg did not affect food intake or growth rate. However it did significantly improve FCR (Table 1). No significant differences in digestibility were observed between any of the laboratory analysed parameters (Table 2).

Table 1 Performance of pigs 7-14 weeks on Control and DeviGuard[®] treatments

	Control	DeviGuard [®]	s.e.m.	P
7 weeks weight (kg)	14.5	14.3	0.24	NS
14 weeks weight (kg)	54.3	54.6	1.32	NS
<u>7-14 weeks</u>				
Feed Intake (g/d)	1311	1268	37.8	NS
Growth Rate (g/d)	812	822	37.6	NS
FCR	1.61	1.54	0.027	<0.01

Table 2 Faecal digestibility of Control and DeviGuard[®] diets offered to 10 week old pigs

	Control	DeviGuard [®]	s.e.m.	P
Dry matter (g/kg)	0.91	0.91	0.025	NS
Oil B (g/kg)	0.91	0.91	0.032	NS
Nitrogen (g/kg)	0.90	0.90	0.017	NS
Ash (g/kg)	0.67	0.67	0.022	NS

Conclusions DeviGuard[®] does significantly improve the FCR of pigs in the growing period. However the mechanism by which DeviGuard[®] exerts this effect is not through changes in feed digestibility measured in this study. Literature and histopathology results would suggest that the observed improved FCR is a result of the improved "health" of the gut.

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Feeding value of fish meal analog as a protein source for starter pigs

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Introduction Young pigs kept intensively, are very sensitive to the source of dietary protein (Maxwell *et al.*, 2003). Many dietary proteins produce allergic reactions in which diarrhoea, reduced growth, and increased mortality can occur (Bimbo and Crowther, 1992). Various protein sources have been tested in early-weaned pig diets in an attempt to overcome these problems and to decrease diet cost. Fish meal analog (FMA), (a product of Mid-South Milling Co., Memphis, TN) based on animal proteins has been reported to have a composition very similar to fish meal, thereby possessing excellent protein and AA source for young pigs (Maxwell *et al.*, 2003). It is less expensive and more readily available. This may therefore provide a high quality protein and AA source for use in starter pig diets at costs lower than those associated with fish meal. This study was therefore conducted to determine the potential of FMA as a replacement for 72% crude protein-imported fish meal (IFM) in diets of starter pigs.

Materials and methods Forty five newly weaned pigs (about 8 weeks of age) were allotted to three dietary treatment groups of 0, 50 and 100% replacement levels of 4kg IFM/100kg diet with FMA on a weight for weight basis. Each treatment was replicated thrice in a completely randomized design, with 3pigs/pen representing a replicate and five of such pens assigned to each treatment. The diets were formulated to contain about 20% crude protein and the pigs housed in concrete floored pens containing feeding and watering troughs for the 42 days duration of the study. Proximate compositions of test ingredients and feed samples were done according to the methods of A.O.A.C. (1990). Blood samples were randomly collected from 2pigs/replicate for the three treatments at the end of the feeding trial for some serum metabolites and haematological studies as described by Adesehinwa (2007) and Mafuvadze and Erlwanger (2007). All the data obtained were subjected to analysis of variance and where statistical significance were observed, the means were compared using the Duncan's Multiple Range (DMR) test (Steel and Torrie, 1980). The SAS Computer software package (1988) was used for all statistical analyses.

Results FMA used in the study contained 70% crude protein, 1.4% crude fibre, 6.5% ether extract, 0.65% methionine, 2.75% lysine, 3.15% threonine and 0.50% tryptophan. The DWG (0.4 - 0.5kg), DMI and Feed:Gain of the starter pigs were not significantly ($P>0.05$) affected (Table 1) by the dietary treatment. The haematological indices (PCV, Hb, RBC and WBC) were also not significantly influenced by the graded levels of IFM replacement with FMA. However, the replacement levels of FMA resulted in a significant reduction ($P<0.05$) in the serum albumin contents that seemed not to be sufficient enough to bring about changes in the serum total protein values of the pigs on the test diets. Changes in albumin content have been reported to be responsible for observed changes in total protein content (Adesehinwa *et al.*, 2007). The FMA replacement of the 4kg IFM/100kg diet resulted in a reduction of between 5.6-11.2% in the feed costs. Consequently, this brought about a resultant significant ($P<0.05$) decrease of 9.2-10.4% in the cost of feed required to gain a kg in weight when fed the FMA diets. Cost of feed per gain has been reported as the most important factor of determining the efficiency of a feed.

Table 1 Performance, cost of feed conversion and blood parameters of starter pigs fed FMA

Parameters	0%FMA	50%FMA	100%FMA	SEM
Daily weight gain (DWG)kg	0.4	0.5	0.4	0.06
Feed:Gain	1.6	1.6	1.7	0.09
% Reduction in cost of feed/kg	-	5.6	11.2	-
Cost of feed/Gain (N/kg)	64.1 ^a	58.2 ^b	57.4 ^b	0.05
% Reduction in cost of feed/gain	-	9.2	10.4	-
Packed Cell Volume (PCV)	39.2	41.6	41.4	0.64
Red Blood Cell (RBC)	724.6	668.8	718.8	17.89
Total Protein (g/dl)	5.7	6.1	5.7	0.14
Albumin (g/dl)	4.6 ^a	4.0 ^b	3.8 ^b	0.13

a, b: Means along the same row having different subscripts differ significantly at $P<0.05$

Conclusion From the results obtained in this study, it could be concluded that FMA has the potential of effectively replacing IFM in diets of starter pigs without adversely affecting the performance and health status of the pigs, both at the 50% and 100% replacement levels, thereby reducing the cost of feeding this class of pigs from weaning to 42days.

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The effect of terminal sire breed on the fatty acid profile of pork

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Introduction The fatty acid profile of pork can be easily manipulated via the diet of the pig. The most common aim is to increase the proportion of unsaturated fatty acids, in particular omega 3 fatty acids. Kim *et al.* (2008) reported that the breed of pig can have an effect on the fatty acid composition of pork. The aim of the current experiment was to investigate the effect of terminal sires of different breed commonly used in Northern Ireland on the fatty acid profile of pork.

Materials and methods All pigs were sourced from the same herd and were offered the same dietary regime from birth to bacon. The finisher ration was a simple wheat/barley/soya mix. Pigs were born to F1 Landrace x Large White dams. Their sire was either 'Tempo' (n=11 (5boars, 6 gilts)), 'Pietrain Austrian' (n=12 (6boars, 6 gilts)) (Piet/Aus), 'Pietrain Belgium' (n=9 (5boars, 4 gilts)) (Piet/Bel) or 'Landrace' (n=7 (4 boars, 3 gilts)) (Land). All pigs were taken to a target live weight of 115kg. Representative chops were taken from the rump end of the loin from each pig after slaughter. The skin was removed from each chop and the adipose tissue was separated from the lean muscle tissue. Representative sub samples of lean (1.0g) and adipose (0.1g) tissue were taken for fatty acid analysis by gas chromatography (GC). Fatty acid methyl esters were prepared by the rapid direct method of O'Fallon *et al* 2007. Internal standards and external methyl ester standards were used for identification and recovery efficiency purposes on the GC. Results were analysed using REML analysis (Genstat Version 11) taking sex effects into consideration.

Results There was no significant difference in the finish weight of pigs which averaged 114.4kg. The adipose tissue from Pietrain Belgium sired pigs had the lowest percentage of total saturated fatty acids (Σ Sat) and the highest percentage of total unsaturated fatty acids (Σ Unsat), total omega 3 (Σ n3) and total omega 6 fatty acids (Σ n6), polyunsaturated fatty acids (PUFA) and omega 6 long chain polyunsaturated fatty acids (LCPUFA n6 respectively) (Table 1). Similarly the lean tissue of Pietrain Belgium sired pigs had the lowest percentage of total saturated fatty acids and the highest percentage of total unsaturated fatty acids, total omega 6 fatty acids and omega 3 long chain polyunsaturated fatty acids (LCPUFA n3). Breed of terminal sire had no significant effect on the percentage of monounsaturated fatty acids (MUFA). There was a higher percentage of omega 3 and 6 long chain polyunsaturated fatty acids in the lean tissue compared with the adipose tissue.

Table 1 The effect of terminal sire breed on the fatty acid profile (% of total fatty acids) of pork from the loin.

Fatty acids:	Adipose Tissue					SED	Sig.	Lean Tissue					SED	Sig.
	Land	Piet/Aus	Piet/Bel	Tempo				Land	Piet/Aus	Piet/Bel	Tempo			
C16:0	24.5 ^b	24.3 ^{ab}	23.0 ^a	23.6 ^{ab}	0.55	*		25.2	24.9	24.5	24.4	0.32	0.089	
C18:0	13.8 ^{ab}	14.5 ^b	12.3 ^a	15.2 ^b	0.79	*		14.1 ^b	14.4 ^b	12.7 ^a	13.4 ^a	0.48	***	
C18:1c9	35.1	34.6	33.9	36.0	0.92	NS		35.5 ^{bc}	35.1 ^{ab}	34.4 ^a	37.1 ^c	0.95	*	
C18:2c9,12	16.4 ^a	16.8 ^a	20.0 ^b	17.3 ^a	1.07	**		13.2	14.0	15.7	13.1	1.03	0.075	
C18:3	1.65 ^a	1.69 ^a	1.93 ^b	1.68 ^a	0.10	*		1.15	1.18	1.26	1.14	0.09	NS	
Σ Unsat	60.1 ^{ab}	59.7 ^a	63.1 ^c	62.3 ^{bc}	1.14	**		58.9 ^a	59.0 ^a	61.0 ^b	60.5 ^b	0.62	***	
Σ Sat	40.0 ^{bc}	40.3 ^c	36.9 ^a	37.8 ^{ab}	1.14	**		41.2 ^b	41.0 ^b	39.0 ^a	39.5 ^a	0.62	***	
Σ n3	1.78 ^a	1.78 ^a	2.07 ^b	1.79 ^a	0.107	*		1.47	1.48	1.68	1.43	0.10	0.094	
Σ n6	17.0 ^a	17.3 ^a	20.7 ^b	17.9 ^a	1.10	**		15.0 ^a	15.7 ^a	17.7 ^b	14.7 ^a	1.12	*	
MUFA	40.7	39.9	39.6	41.9	1.14	NS		41.9	41.2	41.1	43.8	1.22	0.050	
PUFA	19.4 ^a	19.8 ^a	23.5 ^b	20.4 ^a	1.22	**		17.0	17.7	19.9	16.7	1.24	0.053	
LCPUFA n3 [†]	0.12	0.09	0.14	0.11	0.021	NS		0.29 ^a	0.31 ^a	0.41 ^b	0.30 ^a	0.042	*	
LCPUFA n6 [†]	1.17 ^a	1.23 ^a	1.38 ^b	1.28 ^{ab}	0.060	*		2.12	2.24	2.54	2.08	0.183	0.065	

^{a,b,c}Numbers with common superscripts are not significantly different. NS Not significant, * P<0.05, ** P<0.01, *** P<0.001

[†] LCPUFA n3 (C20:5, C22:5, C22:6), LCPUFA n6 (C20:2, C20:3, C20:4)

Conclusion. Terminal sire breed has a significant effect on the fatty acid profile of pork. In the current experiment, the lean tissue of Pietrain Belgium sired pigs had the highest percentage of total unsaturated fatty acids and omega 3 long chain polyunsaturated fatty acids, even though all pigs were offered the same diet. This suggests that different breeds of pig vary in their ability to elongate and desaturate dietary 18:2 n6 and 18:3 n3 to long chain polyunsaturated fatty acids.

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Associations of objective physical measurements of beef meat samples and tenderness assessed by a trained taste panel

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Introduction Meat tenderness is the single most important quality attribute in consumer acceptance studies of meat eating quality. Several mechanical-based techniques have been developed to provide an objective measure of meat tenderness that have good correlation with sensory tenderness, as assessed by trained taste panel. The classic Warner-Bratzler shear force technique (WBSF) has shown correlations with sensory tenderness in the range of -0.39 to -0.77 (Van Oeckel *et al.*, 1999; Shackelford *et al.*, 1999a), whilst the Volodkevitch bite test attempts to imitate the incisor biting action by a compression method. A rapid slice shear force (SSF) test, which uses only one steak and hot meat (significant benefits in a commercial environment), had a stronger correlation with taste panel tenderness scores than WBSF (Shackelford *et al.*, 1999b). The Meat Industry Research Institute of New Zealand (MIRINZ) test results, transformed into categories, was highly correlated (-0.97) with sensory tenderness (Bickerstaffe *et al.*, 2001). The objective of this study was to evaluate the associations between objective physical measures (SSF, MIRINZ and Volodkevitch) and taste panel tenderness scores in beef.

Materials and methods Meat samples of 150 animals, 41 crossbred Aberdeen Angus (AAx) and 43 crossbred Limousin (LIMx) steers, and 32 and 34 crossbred AAx and LIMx heifers were used. One hundred animals were sourced from SAC farms and 50 selected from commercial farms. The *M. longissimus thoracis et lumborum* was sampled from the left side of the carcass at 48 hours post-mortem (pm), dissected into the 11th and 12th ribs cut, a 13th rib cut and the remaining lumbar cut. The SSF test was applied to 3 day aged fresh meat from the 13th rib cut, all other tests were carried out on prior frozen meat. The residue of 13th rib cut was used for the MIRINZ test, in which samples were heated in a water bath to 75 °C and 10 sub-samples of 10 mm by 10 mm cross-section orthogonal to muscle fibre orientation were analysed according to MIRINZ tenderometer protocol. Meat from the 11-12th rib cut was aged at 2°C for 14 days pm and subsequently used for trained taste panel assessment (cooked to a centre temperature of 74°C). The Volodkevitch test was applied to the first 100 mm of the cranial end of the lumbar cut after 10 days ageing. For the Volodkevitch test, a 75mm section of muscle was cooked in an 80°C water bath to a centre temperature of 78°C and 10 blocks cut as for the MIRINZ test protocol. Subsequently, a 25 mm section of the lumbar cut was taken for SSF test on 14 day aged meat. SSF tests were carried out according to the protocol described by Shackelford *et al.* (1999b) with the exception that samples were cooked in a clam-shell grill to a centre cooking temperature of 71°C. Descriptive statistics and Pearson correlations were obtained using Genstat 8.1 and statistical tests of correlations between each test and taste panel were conducted according to Russo (2003).

Results Descriptive statistics and Pearson correlations between objective physical measurements of beef meat samples and sensory tenderness are presented as a correlation matrix in Table 1. Mean SSF at 3 and 14 days pm showed the expected increase in tenderness with increasing maturity of meat. The 3 day SSF and MIRINZ measures showed higher correlations with taste panel tenderness scores but only 3 day SSF was significantly different from Volodkevitch and SSF 14 day results, but not from MIRINZ test. The MIRINZ correlation was not significantly different from either Volodkevitch or SSF 14 day tests. Correlation coefficients of Volodkevitch and 14 day SSF measures are towards the lower level published in the literature.

Table 1 Means, standard deviations (SD), coefficients of variation (CV) and correlations of mechanical and sensory tenderness measurements (units are in Newtons, N, apart from category panel scores which range 1-8)

Test	n	Descriptive statistic			Correlation.(r) *			
		Mean	SD	CV(%)	r (taste)**	r (Volod)	r (MIRINZ)	r (SSF 14)
SSF 3 day PM (N)	150	188.1	70.3	37.4	-0.60 ^a	0.49	0.69	0.50
SSF 14 day PM (N)	150	124.7	32.8	26.3	-0.47 ^b	0.52	0.41	
MIRINZ (N)	131	60.6	23.7	39.1	-0.58 ^{ab}	0.37		
Volodkevitch (N)	150	50.4	14.2	28.3	-0.47 ^b			
Taste panel texture	150	4.8	0.73	15.3				

* All correlation coefficients were significantly different from zero (P<0.001)

** Correlation coefficients with different superscripts are significantly different (P<0.05)

Conclusion The earlier SSF carried out at 3 days pm resulted in a higher association with sensory tenderness than with either of the tests on aged samples. Therefore, SSF measurements recorded early after slaughtering can be used to estimate tenderness of matured meat assessed by a taste panel.

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Potential of NIR spectroscopy for prediction of tenderness of beef sirloin

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Introduction Prediction of eating quality of beef at an early stage *post mortem* would enable selection of carcasses for specific markets. Shackelford *et al* (2004) reported the design of NIR equipment suitable for measurement of larger areas of the muscle and also the prediction of 14 day slice shear force values from NIRRS measurements made at 2 days post slaughter (Shackelford 2005). The aim of the present study was to evaluate the use of equipment of a similar design and manufacture to that used by Shackelford *et al* (2005), to predict Warner-Bratzler Shear force values in carcasses with a smaller range of marbling than found in the USA.

Materials and methods The experiment involved measurement of near infrared reflectance spectra (NIR) of 150 sirloins from bulls, steers and heifers at 2 day *post mortem*. Samples 125-150mm long were obtained from the anterior end of sirloins at 2 days post slaughter and a sub sample taken measurement of ultimate pH (pH_u). The remaining portion was cut into two and NIR reflectance readings taken immediately after cutting (fresh cut surface) over the wavelength range 200 to 1500 nm using a reflectance spectrophotometer fitted with a measuring head specially designed for meat studies (Analytical Spectral Devices Inc, provided by Analytik UK). The samples were allowed to bloom for at least one hour in the cold room. After 1 hour, reflectance measurements were made on the same two surfaces (bloomed spectra). Samples were then vacuum packed and stored in the cold room (2°C) up to 14 or 21 days post slaughter for measurement of Warner-Bratzler shear force (WBSF). Samples were cooked in a water bath and cooking loss determined prior to measurement of WBSF on 12.7 mm cores taken along the direction of the muscle.

The dataset was divided into a calibration set (120 samples) and a validation set (30 samples). Multiple regression analysis was performed on the calibration set and the resulting regression models tested on the validation set.

Results Statistically significant relationships were obtained between NIR spectral data for both fresh cut and bloomed surface and WBSF for both 14 and 21 day aging (Table 1). The percentage variance explained ranged from 25 to 73 % with the best prediction model being obtained between NIR measured on the bloomed surface and the WBSF measured at 21 days aging (73.5% of variance explained). The prediction model based on bloomed surface and WBSF at 21 days aging used 55 variables in the model and is likely to be overfitted. When the prediction model was applied to the smaller validation set the percentage variance explained was lower (Table 1).

Table 1 Prediction models developed for NIR over the range 450 to 1350 nm against WBSF at 14 & 21 days aging

Surface for NIR (number) ^a	Days aged for WBSF	Calibration Set (n=120)				Validation Set n=30 (actual vs predicted)		
		P	% variance explained	RSD	Number of variables	P	% variance explained	RSD
Fresh cut (120)	14	***	25.2	0.45	9	ns	5.6	0.23
Fresh cut (120)	21	***	51.0	0.48	22	**	20.7	0.54
Bloomed (120)	14	***	30.1	0.43	16	**	21.2	0.25
Bloomed (120)	21	***	73.5	0.35	55	**	24.4	0.88

RSD residual standard deviation; ns, not statistically significant (P>0.05); * P,0.05; ** P<0.01; *** P<0.001

Conclusion The NIR equipment used at 2 days post-mortem shows the potential to predict Warner-Bratzler shear force values at both 14 and 21 days *post mortem*. The predictive ability of the different models varies and further statistical evaluation is required to see if more robust models can be developed. If the equipment is to be used on-line then the meat plant will need to decide whether to measure the NIR spectra immediately after quartering or when the carcass has bloomed. Regular checks and update of the calibration would be required.

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Influence of time of measurement post mortem on the near infra-red spectra of beef *Longissimus dorsi* and prediction of tenderness

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Introduction Prediction of eating quality of beef at an early stage *post mortem* would enable selection of carcasses for specific markets. Shackelford (2004) reported the design of near infra red (NIR) equipment suitable for measurement of larger areas of the muscle and also the prediction of 14 day slice shear force values from NIR measurements made at 2 days post slaughter (Shackelford 2005). The aim of the present study was to evaluate the use of equipment of a similar design and manufacture to that used by Shackelford *et al* (2005), to evaluate repeatability and time of measurement of NIR spectra on the prediction of Warner-Bratzler Shear force values.

Materials and methods Samples 55 mm thick were obtained from the anterior end of the sirloin (*Longissimus dorsi*, LD) 3 days post slaughter. Sample collection was undertaken on a number of occasions with 25 to 30 animals being sampled at random each day. A total of 150 steaks were collected. Steaks were taken to the laboratory and the near infrared spectra measured using an Analytical Spectra Devices (ASD) NIR spectrophotometer (Agri Spec) fitted with a special probe for measurements on beef. The reflectance spectra were acquired over the wavelength range 360 to 2300 nm. On all 150 samples a spectral measurement was made on each surface of the steak. On a smaller sample set the measurement probe was replaced 3 times on each surface in similar, but not identical positions, to evaluate the errors involved in sampling. Following spectral measurement the samples were cut in half and vacuum packed and stored until 7 days and 14 days *post mortem*. After these periods NIR spectral measurements were repeated as before and the samples cooked for measurement of Warner-Bratzler shear force (WBSF). The dataset was divided into a calibration set (120 samples) and a validation set (30 samples). Multiple regression analysis was performed on the calibration set and the resulting regression models tested on the validation set.

Results In the spectral region between 600nm and 1100 nm around 40% of the variability in spectra between samples can be explained, at both lower and higher wavelength regions this decreased to 20%. Prediction models between NIR spectra measured at 3,7 or 14 days and WBSF values at 7 & 14 days were statistically significant (Table 1) and explained between 33 & 63% of the variance. When tested on the validation set (30 samples) the variance explained was lower and ranged from 7.4 to 35.2 % of the variance explained (Table 1). The models included a large number of variables and may be overfitted.

Table 1 Prediction models developed for NIR measured at 3, 7 & 14 days post slaughter, over the range 450 to 1350 nm, against WBSF at 14 & 21 days aging.

Time of measurement		Calibration Set (n=120)				Validation Set n=30 (actual vs predicted)			
Days NIR spectra Measured	Days aged for WBSF	Significance	% variance explained	RSD	Number of variables	Significance	% variance explained	RSD	
3	7	***	63.3	0.46	40	***	35.2	0.66	
3	14	***	53.4	0.46	47	ns	7.4	0.41	
7	7	***	56.4	0.51	23	***	49.4	0.47	
7	14	***	33.2	0.55	14	***	18.8	0.36	
14	14	***	52.7	0.46	38	***	23.8	0.53	

RSD residual standard deviation; ns not statistically significant ($P > 0.05$); * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Conclusion The results show that positioning of the reflectance probe is critical and the region of the meat selected for measurement must be consistent. The prediction models developed show that NIR over the region 450 to 1350 nm has the potential to be used as a potential method for selection of tender carcasses. Further work is required to fully develop the prediction.

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On-line prediction of the fatty acid content of beef meat by near infrared reflectance spectroscopy

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Introduction The amount and fatty acid (FA) composition of beef intramuscular fat (IMF) are key factors that influence technological and sensory quality, especially shelf-life (lipid and pigment oxidation) and flavour. Furthermore, consumers are interested in the fat composition of meat, as nutritional guidelines are recommending a lower saturated FA (SFA) intake due to its association with cardiovascular diseases. The amount and composition of ruminant IMF, which depends on factors such as the genetic origin of the animals, feeding regime, age or live weight, influences the final quality of the product, which also explains the increasing interest in defining the FA profile of meat. However, quantitative chemical techniques for the determination of FA involve extraction of total lipids and determination of FA methyl esters by gas chromatography, so that this procedure is costly, time-consuming and generates hazardous waste. The use of near infrared reflectance spectroscopy (NIR) is increasing in food analysis because it offers several advantages over conventional methods, giving fast, non-destructive, clean and cost effective measurements. Therefore, the aim of this study was to test the on-line estimation of the concentration of major individual FA (C16:0, C18:0 and C18:1) and main groups of FA (SFA, monounsaturated FA (MUFA) and polyunsaturated FA (PUFA)) of beef IMF using NIR, by direct application of a fibre-optic probe to the *M. longissimus thoracis* with no prior sample treatment.

Materials and methods Cross-bred steers and heifers (n = 194; sired by either Aberdeen Angus or Limousin bulls) were used in this three-year study. The animals were slaughtered in 11 batches from autumn 2006 until spring 2008 at an average carcass weight and age at slaughter of 335 (SD = 30.3) kg and 584 (SD = 26.2) days, respectively. NIR measurements were taken from the left side carcass *M. longissimus thoracis* (13th rib region) at 48 hours *post mortem* (pm), by means a NIR spectrophotometer (Qualispec Pro, ASD Inc., Boulders, Colorado) provided with a sampling fibre-optic probe. Meat samples were scanned at every 1 nm over the NIR spectral range (350 to 1,800 nm). The absorbance data were subjected to multiplicative scatter correction and/or derivatives. Full cross-validation was applied and the best equation was selected on the basis of minimising the standard error of cross-validation (SE_{CV}). One inch thick steak from the *M. longissimus lumborum* from the cranial end was vacuum packed at 48 hours pm and frozen immediately until the fatty acid composition was determined. Fatty acid analysis was carried out by direct saponification as described in detail by Teye *et al.* (2006). Samples were hydrolysed with 2M KOH in water:methanol (1:1) and the fatty acids extracted into petroleum spirit, methylated using diazomethane and analysed by gas liquid chromatography.

Results From the 194 beef samples, the highest correlations between NIR absorbance data transformed to second-order derivative spectra and major individual FA (C16:0, C18:0 and C18:1) were located at wavelengths of 950, 1100-1400 and 1650-1780 nm, which correspond to absorption by C-H bonds related to long C-H chains of fatty acids (Figure 1). Hence, it is possible that information related to the FA composition of the beef samples could be explained using absorbance data. In order to validate this hypothesis, prediction equations were developed. The prediction results using the fibre-optic probe showed partial least squares correlations (SE_{CV}, g.100g⁻¹ muscle) for C16:0, C18:0, C18:1, SFA, MUFA and PUFA of 0.784 (0.22), 0.684 (0.10), 0.785 (0.33), 0.742 (0.35), 0.797 (0.37) and 0.488 (0.03), respectively.

Figure 1 Correlation coefficients (r) between individual FA content and the absorbance data of the second-order derivative spectra of beef samples

Conclusions The moderate to high correlations between NIR measurements and FA content indicate that NIR measurements recorded in the abattoir could be used for the prediction of FA content of beef meat. Saturated and monounsaturated FA were more accurately predicted than polyunsaturated FA. In summary, the results of this study suggest that NIR spectroscopy could be used on-line as a fast and relatively inexpensive estimation of FA in beef, which may be exploited by the abattoir for a value-based marketing system or by breeding organisations for genetic improvement of meat quality.

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Associations between beef density by X-ray computed tomography, intramuscular fat and fatty acid composition: preliminary results

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Introduction X-ray computed tomography (CT) scanning makes use of the different rates at which the tissues attenuate X-rays depending on their densities. CT muscle density (MD) is the average pixel value for this tissue in the CT images and is related to real density of the tissue, which depends on its chemical composition. One of the components determining muscle density is intramuscular fat (IMF). Studies in sheep have shown that MD, measured *in vivo*, was phenotypically and genetically correlated to IMF and to fatty acid (FA) composition in Scottish Blackface lambs (Karamichou *et al.* 2006). Intramuscular fat and FA are important traits to measure due to their association with the nutritional value and eating quality of meat. A novel automatic image analysis for spiral CT scans (SCTS) of beef primal cuts allows very accurate estimations of primal cut and carcass composition with R^2 of 0.90 to 0.99 (Navajas *et al.*, 2008). The aim of this study was to investigate the associations of MD of beef primal cuts with IMF and FA profile in Aberdeen Angus (AA) and Limousin (LIM) cattle, based on the CT thresholds estimated as part of the development of the image analysis described by Navajas *et al.* (2008).

Materials and methods Data were collected on 85 AA and 105 LIM crosses of two sexes (heifers and steers) slaughtered in 11 batches in autumn-winter of 2006 to 2008. Twenty primal cuts per left carcass side were obtained and vacuum packed 48 hours after slaughter and then CT scanned individually. A one inch thick steak from the cranial end of the M. *longissimus lumborum* was used for fatty acid composition as described in detail by Teye *et al.* (2006). Total IMF content (g/100g muscle) was calculated gravimetrically as total weight of FA extracted whilst SFA, MUFA and PUFA (g/100g muscle) were the sum of the saturated, monounsaturated and polyunsaturated FAs identified, respectively. The percentages of these groups of FA were calculated as the ratio of SFA, MUFA and PUFA to the sum of the total fatty acids identified. MD was calculated as the average of the pixel values (HU), within the CT thresholds for muscle, of the approx. 55 contiguous slices 8-mm thick contained in the SCTS of the sirloins. The Pearson correlation coefficients (r) were calculated between MD and IMF and FA traits. Comparisons between r were performed based on the z-transformation and the u-test (Rasch *et al.*, 1978).

Results Means and standard deviations (SD) of MD, IMF and its FA profile, as well as the r between MD with IMF and FA content, are presented in Table 1. The r were not significantly different between breeds ($P>0.05$) Negative r between MD and IMF content were observed in both breeds, as expected because fat is less dense than the muscle fibre fraction. The magnitudes are in agreement with the phenotypic r reported in sheep (range -0.57 to -0.72; i.e. Karamichou *et al.*, 2006).

Table 1 CT beef density, intramuscular fat and fatty acids in cattle

Trait	AA crosses			LIM crosses		
	Mean	SD	r	Mean	SD	r
MD	69.12	1.5	--	70.09	1.72	--
IMF	2.73	0.96	-0.66	2.12	0.89	-0.55
SFA	1.33	0.42	-0.64	1.06	0.40	-0.55
MUFA	1.42	0.47	-0.64	1.13	0.44	-0.55
PUFA	0.18	0.02	-0.18 ^{ns}	0.18	0.03	0.04 ^{ns}
%SFA	41.07	2.19	-0.15 ^{ns}	40.40	2.50	-0.28
%MUFA	43.60	2.94	-0.30	42.48	2.82	-0.26
%PUFA	5.95	1.91	0.58	7.37	2.23	0.57

^{ns} values not different from zero ($P>0.05$)

MD comprised of the densities of all muscles in the sirloin, not just the section of the *longissimus lumborum*, where FA composition was analysed. The r for SFA and MUFA contents were similar to that for IMF content, probably because each of these FA groups were more than 40% of the fat in the muscle. The low content of PUFA, compared to SFA and MUFA may explain the lack of significant associations with MD. The similar relationships between FA and IMF could explain the reduced opportunity to manipulate %SFA and %MUFA, as indicated by their much lower r values. The moderate r of %PUFA implies that higher values of MD (lower IMF) were related to higher %PUFA.

Conclusion Muscle density of beef primal cuts measured by CT was moderately correlated to IMF content, and its FA composition in both AA and LIM crosses. This implies that the CT tissue thresholds estimated to maximise the accuracy of beef carcass composition, may become a useful tool in breeding programs if genetic correlations are of similar or higher magnitude to the phenotypic estimates obtained here. Further studies on the association of MD with beef eating quality and the genetic parameters are needed.

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X-ray computed tomography accurately assesses beef carcass composition

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Introduction X-ray computed tomography (CT) scanning of live animals and carcasses provides a very accurate assessment of the weights of fat, muscle and bone in pigs (Dobrowolski *et al.*, 2003) and sheep (Johansen *et al.*, 2007). Although the limiting size of the CT gantry prevents CT scanning of live beef cattle, beef primal joints are small enough to be scanned. Navajas *et al.* (2008) explored the use of spiral CT scanning (SCTS) to quantify beef carcass composition. The automatic image analysis developed provided very accurate assessments of primal and carcass composition (Navajas *et al.*, 2008). The objective of this study was to evaluate the accuracy of this novel method for assessing beef primal cut and carcass composition in an independent dataset.

Materials and methods This validation study was based on 462 primal cuts of 8 Aberdeen Angus (AAx) and 14 Limousin (LIMx) crossbred steers (average carcass weights: AAx, 353 kg; LIMx, 359 kg). The estimation dataset was described by Navajas *et al.* (2008). Individual SCTS of each primal, containing contiguous 8 mm-thick slices, were collected. These primal cuts were prepared 48 hours after slaughter and vacuum packed. After CT scanning, primal cuts were fully dissected into fat, muscle and bone. Image analysis was carried out based on the thresholds previously estimated (Navajas *et al.*, 2008). The weights of muscle, fat and bone of the primal cuts were predicted, based on tissue areas and densities and the thickness of slices in the SCTS: Tissue weight = Σ tissue area $\times$ slice thickness $\times$ weighted average density of tissue, where weighted average density = Σ (area $\times$ 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 $\times$ 0.00106) + 1.0062 (Fullerton, 1980). Bone density assumed in these calculations was 1.55 g/cm^3 . Total carcass composition was calculated by adding the tissue weights of all primal cuts measured by CT or dissection. Accuracy of primal cut composition in the validation dataset was measured using the adjusted coefficient of determination (R^2) and the residual mean squared error (RMSE). The same parameters were used to evaluate the association and accuracy for total carcass composition based on the complete dataset (44 carcasses). In all cases, a 1:1 association between tissue weight by dissection and CT was assumed.

Results Means (M) and standard deviations (SD) of primal cut and carcass tissue weights (kg) measured by CT (M_{CT} , SD_{CT}) and dissection (M_D , SD_D), as well as the corresponding R^2 and RMSE, are presented in Table 1. The weights of the tissues measured by CT and dissection were very similar. Average differences ranged from -10 to 60 g, and 10 and 400g for primal cut and carcasses, respectively.

Table 1 Beef primal cut and carcass composition: associations between CT and dissection (tissue weights in kg)

Primal cut composition	M_{CT}	SD_{CT}	M_D	SD_D	R^2	RMSE
Fat	1.72	1.00	1.71	0.90	0.92	0.25
Muscle	5.29	3.28	5.34	3.24	0.99	0.32
Bone	1.29	0.78	1.35	0.74	0.97	0.14
Carcass composition	AV_{CT}	SD_{CT}	AV_D	SD_D	R^2	RMSE
Fat	35.40	7.42	35.61	6.71	0.96	1.28
Muscle	110.23	11.40	110.65	10.8	0.96	2.28
Bone	26.54	1.59	26.53	1.6	0.95	0.37

methods used in beef to assess carcass composition (i.e. VIA, Allen and Finnerty, 2001).

The results obtained in this validation study show high accuracies of CT scanning with values of R^2 between 0.92 and 0.99, confirming the values obtained when the CT thresholds were estimated (Navajas *et al.*, 2008). Strong associations between CT and dissection weights were also found for total carcass composition as indicated by the high R^2 values and low RMSE values (Table 1). These values are similar to, or better than those obtained using CT in other species (i.e. Dobrowolski *et al.*, 2003), and better when compared to other alternative

Conclusion The automatic CT image analysis developed allows the faster delivery of accurate assessment of beef carcass composition and with lower costs than physical dissection (traditional reference method), without damaging or devaluing the carcasses. This information is very valuable for research, and potentially for breeding programmes and beef industry benchmarking.

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Front face fluorescence spectroscopy as a rapid non-invasive tool for determining diet composition in free-ranging ruminants

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Introduction Quantification of the diet consumed in free-ranging ruminants is essential to manipulate both agricultural performance and environmental impact. A common non-invasive approach for determining diet composition is based on using long-chain, saturated hydrocarbons (n-alkanes) as markers (Dove and Mayes, 1996). However a major drawback of using this approach is the time and related expense associated with extracting these compounds. Spectroscopic imaging is a rapidly evolving research area, with the potential to provide real-time solutions. Previous applications have shown its ability to detect chlorophyll and its breakdown products in faeces as markers of contamination in carcasses to reduce pathogen load entering the food chain (Ashby *et al.* 2003). Chlorophyll is ubiquitous in green plants and thus livestock diets. During digestion in the gut, chlorophyll is only partially degraded to coloured and fluorescent intermediates: phaeophytin, chlorophyllide, phaeophorbide and pyropheophorbide derivatives of chlorophylls a and b. It is these compounds that are detected by spectral imaging of the faeces, and comparisons of spectral wavelength profiles may provide comparative information about the animals' diet.

Materials and methods Welsh Mountain sheep (12) previously grazed on un-improved hill areas in mid-Wales were adapted to indoor pen feeding and offered 20% heather mix (H; predominantly *Calluna vulgaris*) : 80% hill grass mix (HGM; predominantly *Molinia caerulea*, *Juncus* spp, *Festuca* spp and *Bryophyta*) on a DM basis for two weeks. Four sheep were then allocated at random to each of 3 diets containing different ratios of H:HGM: Low (10% H: 90% HGM); Medium (20% H: 80% HGM) and High (30% H: 70% HGM). Diets were offered at 700 gDM/head/d in a single feed given at 0930 for 2 weeks and all refusals measured and speciated. In the second week total daily faecal output was recorded and a sub-sample bulked for each individual animal across the week, retained at -20°C. Fluorescence emission spectra were measured directly on the faeces. The fluorescence emission spectra were measured with excitation at 382 and 430 nm, using an optical bench system. The excitation light was generated by a 300 W Xenon light source (Oriel 6258, Oriel Corporation, Stratford, CT) and passed through a 10 nm bandwidth interference filter (Oriel 59920) and (Oriel 59295). The light was directed onto the samples at an angle of about 45°. The spectra were collected by an imaging spectrograph (Acton SP-150, Acton Research Corporation, Acton, MA) connected to a sensitive charge coupled device (CCD-camera) (Roper Scientific NTE/CCD-1340/400-EMB, Roper Scientific, Trenton, NJ). Cut-off filters at 400 nm (for the 382 nm excitation) (Melles Griot 03FCG049) and 475 nm (for the 430 nm excitation) (Melles Griot 03FCG068) were positioned in front of the spectrograph slit to suppress excitation light reflected from the samples. Exposure time was 10 and 5 sec for excitation at 382 and 430 nm, respectively. The temperature of the samples was 4 °C. All the samples were measured twice and an average was used in the analysis. The resulting spectra were analyzed using PCA (Principal Component Analysis).

Results Figure 1 shows the score plot for the first and second principal component (PC 1 vs. PC 2) which accounted for 98% and 1% of the variability in the data, respectively. The figure clearly shows clustering according to heather allocation in the diet.

Figure 1 PCA cluster for the fluorescent spectra of sheep faeces on diets containing three levels of heather.

Conclusions Front face fluorescence spectroscopy offers a tool for rapidly screening numerous faecal samples to explore potential differences in diet composition, and pin point samples for more detailed, but labour-intensive, analyses.

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Genetic parameters for carcass dimensional measurements from Video Image Analysis and their association with conformation and fat class scores

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Introduction Automatic technologies based on video image analysis (VIA) to assess lamb carcass value offer an accurate measure of weight of individual carcass components (Rius-Vilarrasa *et al.*, 2008). In addition, VIA systems offer the possibility to record linear and area traits (dimensional measurements) on the carcass. Linear measurements on the body and carcass have been suggested by several authors as good indicators of live weight, conformation score and production traits in beef and sheep (Maiwashe *et al.*, 2002; Janssens *et al.*, 2004). Therefore, the aims of this study were (1) to estimate genetic parameters for VIA dimensional measurements (VIA-DM) and (2) to investigate the associations between VIA-DM with carcass conformation and fatness.

Materials and methods Crossbred lambs (n = 630) 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. Lamb carcasses were classified under the MLC EUROP carcass classification scheme for conformation and fatness (MLC-CS) and then VIA scanned to obtain carcass VIA-DM, i.e. lengths (L), widths (W) and areas (A). Genetic parameters for MLC-CS and VIA-DM were estimated by fitting a multivariate animal model using restricted maximum likelihood (REML) as implemented in ASReml (Gilmour *et al.*, 2004). Fixed effects included, when significant, were batch (year of birth, sex and farm), sire breed and dam age. When significant, finishing age was included as a covariate.

Results Heritability estimates for subjective carcass traits from the MLC-CS were low (0.10 both for conformation and fat) and heritabilities for VIA-DM ranged from moderate to high (0.25 – 0.46; Table 1). Phenotypic correlations between MLC-CS and VIA-DM were in general low (0.01 – 0.51), but genetic correlations (in absolute value) were higher both for conformation (0.07 – 0.81) and fat (0.04 – 0.73), although for fat most of the correlations were not significantly different from zero. Genetic correlations among VIA-DM were generally high as shown in Table 1.

Table 1 Estimates of heritabilities (on the diagonal), phenotypic (above) and genetic correlations (below the diagonal) (standard errors) for the VIA-DM[§] which explained most of the variability on the VIA estimated weight of primal joints[†]

	L3	W2	W5	W7	A1	A3
L3	0.46 (0.11)	0.31	0.58	0.62	0.78	0.86
W2	0.51 (0.17)	0.36 (0.11)	0.61	0.47	0.75	0.50
W5	0.42 (0.16)	0.47 (0.17)	0.39 (0.10)	0.59	0.80	0.68
W7	0.90 (0.08)	0.82 (0.11)	0.50 (0.18)	0.27 (0.10)	0.65	0.81
A1	0.91 (0.05)	0.82 (0.08)	0.61 (0.13)	0.98 (0.05)	0.34 (0.10)	0.80
A3	0.95 (0.04)	0.61 (0.17)	0.67 (0.21)	0.96 (0.04)	0.90 (0.06)	0.25 (0.09)

[§] L3 - Total length heel to shoulder, W2 - Minimum breast width, W5 - Maximum leg width, W7 - Minimum waist width, A1 - Back area of the carcass minus legs and A3 - Side area of the hind legs

[†] Heritabilities on diagonal, phenotypic correlations above diagonal and genetic correlations below diagonal. Standard errors for phenotypic correlations were less than 0.04.

Conclusions Heritability estimates for subjective and objective measures of carcass traits indicate that more rapid rates of genetic progress could be achieved by using VIA-DM compared to using MLC-CS. Carcass conformation could be improved by genetic selection using VIA-DM. However the associations between VIA-DM and carcass fatness could not be elucidated due to high standard errors. In general, moderate to high phenotypic and genetic correlations were found between the VIA-DM *per se* which will result in a correlated response to selection. This implies that selection towards larger (longer, L3 and wider, W5) hind legs will also increase the size (A1, A3) of the whole carcass. Further analysis would be required to elucidate the optimal way to use this VIA information in the current breeding programs which aim to increase lean meat yield on the most valuable cuts while reducing the overall carcass fatness.

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The behaviour of finisher pigs of three genotypes during routine handling using standard or modified weighing apparatus

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Introduction The effect of stress on pigs prior to slaughter is of welfare and economic importance, and can negatively impact on meat quality. Strategies to reduce the stress of finishing pigs have previously tended to concentrate on a single breed. This research aims to target this lack of knowledge by assessing differences between genotypes when being handled.

Materials and methods In Trial one (T1), 478 slaughter weight pigs ($103 \pm 0.39\text{kg}$) were weighed 24h prior to slaughter (n= Hampshire :172, Large White:161, Pietrain:145) using a standard electronic metal pig crate surrounded by metal hurdles. Pigs were herded to the weighing area and individually weighed. Pigs were timed for the duration of the weighing process, from the selection of a pig to its exit from the crate. The pig was restrained in the crate whilst its ear tag was read and a new tag inserted. The crate was then opened and the pig allowed to leave voluntarily. If the pig did not start to move immediately, it was encouraged by research staff. Behavioural reactions of the pigs were scored 1 (easy/calm) to 5 (difficult/fearful) over four stages of the weighing process: Loading; Behaviour in the Crate; Tagging and Exiting. Vocalisations were also recorded.

Trial two (T2): 210 pigs were used; the pigs were balanced for genotype. The hurdles immediately surrounding the weigh crate were painted brilliant white. This aimed to reduce the fearful response of pigs and increase their willingness to enter the crate by brightening target areas (Tanida *et al.*, 1996). The scoring system remained the same. Analysis was performed using General Linear Model ANOVA procedures in Minitab 15.0.

Results T1: Significant effects were apparent between genotypes in loading ease ($P < 0.01$), time taken to weigh ($P < 0.001$), and exit from the weigher ($P < 0.05$). A sex and genotype interaction was evident in the behaviour in the weigher (Table 1 $P < 0.05$).

Table 1 Mean behaviour score in weigher and SE.

	Hampshire Mean SE	Large White Mean SE	Pietrain Mean SE
Male	1.58±0.06	1.71±0.09	1.63±0.08
Female	1.83±0.06	1.58±0.07	1.60±0.08

T2: Loading ease differed between the genotypes in this trial ($P < 0.001$), as did exit from the weigher ($P < 0.05$) and the time taken to weigh ($P < 0.01$). Tagging of females was harder than males ($P < 0.05$, 1.59 ± 0.07 , 1.45 ± 0.07). Pietrain pigs vocalised more frequently than Hampshires or Large Whites and the number of vocalisations differed between genotypes ($P < 0.05$, 1.93 ± 0.03 , versus 1.82 ± 0.07 and 1.75 ± 0.06 , respectively).

T1 vs T2: Genotypic differences were again evident in loading ease ($P < 0.001$). Hampshire pigs were more difficult in T2 whilst Pietrains were easier. Large White showed no significant change.

Tagging of pigs in T2 tended to be easier ($P = 0.065$; T1: 1.65 ± 0.03 ; T2: 1.51 ± 0.05), particularly in male pigs ($P = 0.051$, 1.45 ± 0.07 , 1.59 ± 0.07). The time taken to weigh pigs altered between trials ($P < 0.05$). No genotype specific patterns were found however. Exit from the weigher showed Hampshires as being most difficult in T2, whilst Pietrains were easiest ($P < 0.05$) (Table 2).

Table 2 Genotypic Differences in Handling Scores and Time Taken

Genotype	Trial	Loading Mean SE	Exiting Mean SE	Time Taken(s) Mean SE
Hampshire	1	1.42 0.04	1.17 0.03	46.7 0.80
	2	2.09 0.02	1.36 0.10	51.6 3.38
Large White	1	1.44 0.05	1.16 0.03	41.3 0.74
	2	1.31 0.07	1.17 0.05	42.6 0.95
Pietrain	1	1.67 0.01	1.19 0.04	47.0 1.77
	2	1.21 0.01	1.07 0.06	42.5 2.21

Conclusions There were significant behavioural differences between genotypes during routine handling. This could have an impact on further management strategies, as genotype-specific schemes may need to be implemented. The provision of white painted handling races could improve handling ease, depending on the genotype of pig being produced.

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Influence of mixing on the initiation of piglet feeding and post weaning growth performance

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Introduction Weaning is a stressful period for the piglet, invariably typified by low voluntary feed intake and reduced performance. Moreover, if siblings are separated and mixed with other litters, stress increases and the learned stimulus of the group to feed is disturbed (Brooks *et al.*, 2003). This experiment was part of a larger investigation into the voluntary feed intake of the piglet in the immediate post weaning period. We investigated the difference between mixed litter origin or sibling groups on latency to initiate feeding and performance to day six post weaning. We hypothesised that mixing litters would increase latency to first feeding and reduce growth rate in the first few days after weaning.

Materials and methods Seventy-six piglets (Large White x Landrace) were weaned at $8.0\text{kg} \pm 0.28$ (s.e.m.) and 28.1 ± 0.39 days of age into eighteen mixed sex groups with four-five pigs per pen. Piglets were housed in fully slatted weaner pens (1.7 m^2). Piglets were allocated to either mixed (each individual from a different litter) or litter pens (siblings), balanced for weight and gender. Feeding behaviour was monitored 24 hours a day using 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 piglets was offered *ad-libitum* access to one feed (16.45 MJDE/kg , 1.6g lysine/kg). Piglets were weighed at weaning and six days post weaning. The time in minutes between entering the pen and being actively present at the trough for a feeding bout of >0.5 minutes was defined as the latency to first meal which was log transformed prior to analysis. Data were analysed using GLM procedures of Minitab 12.2 with replicate in the model and weaning weight as a covariate.

Results There was no significant difference between the latency to first meal between mixed or litter treatments (Figure 1), with a mean latency of $379\text{ min} \pm 73.7$ (s.e.m.). However, piglets in litter pens tended to have higher average daily weight gains than piglets in mixed pens ($P = 0.068$) where Litter = $197\text{ g/d} \pm 33.0$ (s.e.m.) and Mixed = $77\text{ g/d} \pm 33.0$ (s.e.m.) (Figure 2).

Figure 1 Effect of weaning group composition on latency to first meal after weaning.

Figure 2 Effect of weaning group composition on average daily gain to day 6 post weaning.

Conclusion This study found no negative consequences of mixing unacquainted piglets on the initiation of feeding post-weaning, in contrast to our initial hypothesis. However our results do suggest a post weaning growth benefit when piglets remained with their littermates indicating that mixing piglets at weaning is disruptive which is in line with our hypothesis. Indeed, Tan *et al.* (1991) stated that mixing piglets at weaning increases stress and aggression which would decrease FCR efficiency and reduce weight gain post weaning. Mixing litters post weaning is common practice in the pig industry in order to 'size' pigs for easier management. However, consideration should be given to alternative mixing strategies at weaning in order to improve piglet welfare and performance during this critical period.

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Influence of drinker design and position on the behaviour of growing pigs

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Introduction A number of different drinker types are available commercially for pigs. These typically include drinkers where water is dispensed into a bowl or consumed straight from a water nipple. The design of drinkers may influence levels of competition at the drinker, and also levels of water wastage. This latter factor has adverse environmental implications and may arise from pigs switching between drinkers during drinking bouts, or using drinkers for 'recreational' purposes. The aim of this study was to assess behavioural differences at the drinker between pigs offered different types of commercially-available drinker.

Materials and methods A total of 720 pigs were weaned at four weeks of age and allocated in groups of 20 to one of six treatments until 10 weeks of age. The treatments consisted of four drinker designs, two of which were tested in two different arrangements. Each group had access to two drinkers and treatments were as follows: (1) Bowl A – together (two bowl drinkers side by side), (2) Bowl A – apart (two bowl drinkers placed 1.5 m apart), (3) Bowl B – together, (4) Bowl B – apart, (5) Bite A and (6) Bite B. Bowl A drinkers (Drik-O-Mat, Egebjerg, Denmark) incorporated a horizontal lever which pigs pushed to release water which was retained in the bowl. Pigs therefore drank water from the bowl (width 15 cm, bowl capacity 100 ml). Bowl B drinkers (Verba, The Netherlands) were designed so that pigs could drink directly from the nipple. However, excess water was also retained in the bowl (width 15cm, capacity 210ml). Average water flow rates were 250 ml/min for Bowl A and 600 ml/min for Bowl B drinkers. Bite A drinkers (Monoflo International Inc, Canada) and Bite B drinkers (Bite Button Valve, Jalmarson – Stingy, Sweden) allowed pigs to access water through biting a nipple or a button attached to a spring-loaded valve. Aside from their mechanism for releasing water the main difference between the two bite drinkers was the flow rate they created (700 vs 1200 ml/min respectively). The bite drinkers had a forked arrangement which resulted in the two drinkers being 30cm apart. Pigs were housed in slatted pens (0.38m² per pig) and had *ad libitum* access to concentrate rations. Behaviour at each of the drinkers was video recorded for one 24 hour period when pigs were four, seven and 10 weeks of age. Instantaneous scans were made of each of the drinkers every hour to record whether or not a pig was using the drinker, the number of pigs apparently queuing to use the drinker, standing close to the drinker (but not queuing), and nosing the floor under the drinker. Both drinkers in a pen were also observed simultaneously for a continuous 10 min period at 0800, 1000, 1200 and 1400 hours. The duration of each drinking bout within these observations was recorded. In addition, the number of times pigs removed their head from the drinker, or switched drinkers during drinking bouts was recorded. The proportion of drinking bouts where pigs left the drinker 'voluntarily', i.e. without being moved or displaced by another pig, was recorded. Data from five of the six replicates of this study were used in analysis (Genstat, Version 6.1). The influence of treatment was analysed by Analysis of Variance, blocked for replicate. The statistical model allowed specific comparisons to be made between different bowl drinker positions (side by side or apart), different bowl drinker designs (A or B), between Bite and Bowl drinkers, and between Bite drinkers A and B.

Results Overall treatment comparisons for selected parameters are presented in Table 1. The proportion of drinking bouts where pigs switched drinkers was significantly higher when bowl drinkers were placed side-by-side rather than apart ($P<0.01$). The proportion of drinking bouts where pigs removed their head from the drinker during a bout was greater with Bite than with Bowl drinkers, and was greater with Bite B than with Bite A drinkers ($P<0.001$). More pigs were observed queuing for Bowl than for Bite drinkers ($P<0.05$), however more pigs were observed standing in close proximity to Bite than to Bowl drinkers ($P<0.001$). In addition, more pigs were observed nosing the ground under Bite than Bowl drinkers ($P<0.001$).

Table 1 Influence of drinker design on the proportion of drinking bouts where pigs switched drinkers or removed their head from the drinker, and the number of pigs observed queuing, standing near or nosing the ground under drinkers

	Bowl A (together)	Bowl A (apart)	Bowl B (together)	Bowl B (apart)	Bite A	Bite B	SEM	P
Switching	0.08 ^{ab}	0.03 ^a	0.12 ^b	0.03 ^a	0.13 ^b	0.07 ^{ab}	0.024	<0.05
Removing head	0.05 ^a	0.04 ^a	0.05 ^a	0.03 ^a	0.14 ^b	0.35 ^c	0.026	<0.001
Queuing	0.04 ^{bc}	0.05 ^c	0.02 ^{ab}	0.03 ^{abc}	0.02 ^a	0.02 ^{ab}	0.009	0.05
Near (not queuing)	0.03 ^a	0.05 ^a	0.01 ^a	0.04 ^a	0.13 ^b	0.15 ^b	0.028	<0.01
Nosing floor	0.01 ^a	0.01 ^a	0.00 ^a	0.01 ^a	0.10 ^b	0.12 ^b	0.025	<0.01

^{a,b,c}: Means in the same row without a common superscript differ significantly

The average length of drinking bouts, or the average proportion of scans where pigs were observed using drinkers did not differ significantly between treatments. However, more pigs left drinkers 'voluntarily' with Bowl B than Bowl A drinkers ($P<0.05$).

Conclusions Placing drinkers apart rather than side-by-side reduced switching between drinkers and could therefore reduce water wastage. Increased numbers of pigs gathered round, and nosing the ground under Bite rather than Bowl drinkers suggest that these drinkers were used for recreational purposes to a greater extent. This may also have been reflected in the fact that pigs removed their head from the drinker during drinking bouts more frequently with Bite than with Bowl drinkers.

Responses to multiple chain enrichment in post weaned pigs

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Introduction The social stressors associated with the abrupt weaning process on commercial farms can result in significant welfare issues for pigs; including behavioural problems and aggression, decreased immunological responses, reduced live weight gain and efficiency of feed conversion (van de Weerd and Day, 2008). Slats and slurry systems in commercial housing systems for grower pigs provide a barren environment which may exacerbate these problems. Enriched environments have been shown to reduce aggression in pigs, particularly where devices that have manipulative and destructive properties are used (Scott *et al.*, 2007). However, studies often include a single device that does not permit all animals to interact with the enrichment at any one time. The aim of the present study was to investigate the effects of multiple metal chain enrichment on levels of aggression in growing pigs.

Materials and methods Pigs (n=240) from 24 litters were selected at 28 days of age (weaning; day 0), and split into 12 groups of 20 pigs (two litters per pen); six control groups (CG) and six enhanced enrichment groups (EEG). Pigs were housed in pens (0.4m² per pig) with slatted floors within an environmentally controlled building (ambient temperature approximately 25°C). CG pigs were provided with a single chain (0.6m length, 5mm link thickness) per pen suspended to a height 0.25m above the pen floor (one chain per 20 pigs). In the EEG pens six similar chains were suspended across the pen at 0.5m intervals to avoid sleeping and feeding areas (one chain per 3.3 pigs). All pigs were offered pelleted concentrate feed *ad libitum* throughout the study. Injuries were scored using the seven point scale (0 – no wounds, 6 – severe, infected wounds) developed by Gallois *et al.*, (2005) on days one, three, five, seven, 14, 21 and 28 as a measure of aggressive behaviour within the groups of pigs. Live weights were recorded on days zero, seven, 14, 21 and 28. These weights were used to calculate daily live weight gain (DLWG) over the four week period. The interaction of the pigs with the enrichment device was recorded using intermittent scan-sampling, at five minute intervals for 60 minutes on days one, three, five, seven, 14, 21 and 28. Injury scores, live weights and daily live weight gains (means for pens) were statistically analysed using repeated-measures analysis of variance. The incidence of interaction with the enrichments were statistically analysed using the Chi Square Test.

Results Mean injury scores (Figure 1) were significantly lower ($P < 0.001$) in the EEG pigs throughout the study period (0.73 ± 0.15 versus 1.65 ± 0.21 respectively; mean $\pm$ SEM). During the first seven days post weaning, injury scores in the EEG were lower than among the CG pigs, falling to a mean score of less than 0.5 by day 14. Interactions with the chains were greater ($P < 0.001$) in the EEG throughout the 28 day study; although there was a decrease in interactions with time in both treatment groups. During the first week post weaning interactions with the chains were higher in EEG pigs. There was no significant difference in either live weight (final live weight 17.0 ± 0.69 vs 16.7 ± 0.75 kg; CG vs. EEG) or DLWG (overall DLWG 0.31 ± 0.02 vs 0.33 ± 0.02 kg/d; CG vs. EEG) among the two treatment groups throughout the study.

Figure 1 Mean ($\pm$ SEM) post weaning injury scores for control and enriched pigs

Conclusion Providing chains as a multiple enrichment device to growing pigs increases interaction with the enrichment and reduces injury and aggression particularly during the immediate post weaning period. However, the fall in the frequency of interaction with the chains over the study period indicates that habituation occurs with time post weaning.

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Effect of a compensatory growth feeding regime on performance and gross carcass characteristics in growing / finishing pigs

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Introduction Compensatory (or ‘catch-up’) growth is an accelerated rate of weight gain in animals allowed *ad libitum* access to feed after a period of restricted feeding such that these animals reach the weight of those control animals fully fed (Critser *et al.*, 1995). There are conflicting reports on the ability of pigs previously fed on a restricted basis to compensate completely in terms of performance and gross carcass characteristics once re-fed. The compensatory growth index (CDI) is calculated as the ratio of the difference between weight variation at the end of restricted and compensatory growth periods, respectively, relative to the variation at the end of the restricted growth period alone (Hornick *et al.*, 2000) thereby indicating whether the animals have been able to compensate during the period of suggested catch up growth. The hypotheses of the trial reported were that animals re-fed following feed restriction would achieve the liveweights and gross carcass composition of a parallel control group fed *ad libitum* throughout.

Materials and methods Female pigs (n=48; initial liveweight 24.6 ± 0.68 kg) were individually housed in a controlled-environment experimental growth building. A standard grower/finisher meal-based diet was offered (DE 13.9MJ/kg; CP 165g/kg; lysine 10.42g/kg; methionine+cysteine 5.9g/kg; threonine 6.3g/kg fresh weight). Following an acclimatisation period of 14 days, animals were either *ad libitum* (Group One) or 0.70 of *ad libitum* intake (Group Two) from 74 until 114 days of age (Period One; 40 days). Feed intake (FI) for Group One (*ad libitum*) was recorded weekly and data, in conjunction with weekly liveweights, were used to calculate the feed intake of Group Two (restricted). Subsequently 16 animals (32 having been slaughtered, with data for only 16 employed in the current summary) were fed *ad libitum* until 156 days of age (Period Two; 42 days) when they were slaughtered. Daily liveweight gain (DLWG) was estimated as the slope of the linear regression of time (days) against liveweight (kg). Feed conversion ratios were estimated from daily FI (DFI) and DLWG. Sixteen animals were slaughtered at the end of Period One (Slaughter One; 8 per treatment) and 16 at the end of Period Two (Slaughter Two; 8 per treatment). Standard carcass data were obtained. The statistical model was a Group One Group Two comparison; data were analysed by one-way ANOVA separately for each period.

Results

The initial liveweight of pigs following the two-week acclimatisation period was 33 vs 34 kg (SED 1.0, $P=0.119$). Performance data in Period One were as expected with Group Two animals consuming less food. DLWG for Group Two was 72.8% that of Group One and FCR for Group One was 27.2% better than Group Two. In Period Two, Group Two pigs grew 12.9% faster (with numerical improvements for FI and FCR) thereby indicating compensatory growth. At the end of Period One Group Two pigs had lower CW, but no significant difference in the dissected mass of specific muscles. By the end of Period Two, CW and dissected muscle mass were not significantly different although Group One pigs had higher KO%.

Table 1 Effect of treatment on performance and gross carcass quality

Factor	Period 1				Period 2			
	One	Two	SED	P	One	Two	SED	P
DLWG kg	0.92	0.67	0.119	<0.001	1.08	1.24	0.055	0.014
DFI kg	2.83	2.27	0.111	<0.001	2.94	3.04	0.192	0.616
FCR	3.07	3.35	0.117	0.002	2.72	2.48	0.163	0.165
KO %	76.2	75.7	0.67	0.438	82.2	80.0	0.84	0.019
CW kg	55	48	3.1	0.035	95	92	4.5	0.534
LD kg	1.61	1.65	0.050	0.387	2.92	2.84	0.081	0.243
PM kg	0.34	0.34	0.022	0.841	0.59	0.6	0.035	0.895
ST kg	0.31	0.31	0.022	0.984	0.51	0.51	0.031	0.928

[†], Analyses for *L. dorsi* (LD), *P. major* (PM) and *semitendinosus* (SM) employed CW as a covariate.

Conclusions The CDI was 0.90, which was within the range reported by Hornick *et al.*, 2000, who indicated that the expected range is between 0.5 and 1.0, with 1.0 being rarely observed. Although compensatory growth did take place the data suggest that in the pigs that were restricted then *ad libitum* fed the compensatory response was linked partially to greater visceral mass (as suggested by their lower KO%).

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The pigmeat supply chain: pre-assessment for a Life Cycle Inventory

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Introduction Formulating the inventory of relevant commodities to assess the life cycle of goods or services (LCI) is highly demanding on time and resources (Suh *et al.*, 2004). Collected information is not always satisfactory to take account of all possible sources of environmental burdens (E-burdens) produced in the commodity supply chain. Several pre-assessment methods have been proposed to serve this function, although these have identified limitations; lack of previous experience and use of subjective cut off criteria are the most frequent weaknesses found (Suh, 2006). An objective pre-assessment method was developed as part of a life cycle analysis (LCA) for different pigmeat supply chain (PSC) scenarios.

Materials and methods A database of E-burdens linked to the economic sectors in the U.S. (Green Design Institute, 2007) was used to track E-burdens produced for ten different processes and fifteen E-burden indicators modelled for the PSC. Two assumptions were necessary: that the ten PSC processes modelled, and the economic sectors in the U.S., are representative of standard PSCs. The database used contains a series of Economic Input Output matrix tables that identify the E-burdens linked to the economic participation of all industrial sectors in an economy that supply raw materials when a specific sector demands commodities. E-burdens were expressed as percent of contribution of sectors per indicator. Economic sectors that contributed five percent or more per indicator were included.

Results Between 20 and 30 sectors (for example: power generation and supply, agrochemical manufacturing, truck transportation) were important contributors to E-burdens in each PSC process. By investigating the principal commodities produced by these sectors, decisions could be taken about inclusion in the LCI. A system boundary was then drawn to include the sectors which participate in the supply chain flow. Most of the E-burdens arose from the production or transformation of raw materials (upstream). Only for three indicators (CO, NO_x and N₂O) was the use of commodities within the PSC processes (within-PSC) the principal source of E-burdens. Lead from industrial processes and CH₄ from pig manure fermentation were principal e-burdens from downstream (waste disposal). The use of energy, CO₂ emissions and toxic releases were strongly related to fuels consumed and were highly dispersed among all sectors. Nearly a third of total burdens were attributable to minor sectors with less than 5% contribution (Table 1).

Table 1 Percentage distribution of environmental indicators for the pigmeat supply chain (PSC)

E-burden Indicator		Pigmeat supply chain flow			
		Up-stream	Within-PSC	Down-stream	Minor sectors (<5%)
Air pollutants, %	SO ₂	72	13	0	15
	CO	38	45	0	17
	NO _x	33	49	0	18
	VOC	47	28	0	25
	Lead	56	8	29	7
	PM ₁₀	64	15	3	18
Greenhouse gases, %	CO ₂	41	30	0	29
	CH ₄	63	10	19	8
	N ₂ O	30	62	0	8
	CFCs	95	3	0	2
Energy	%	38	30	0	32
Toxic releases, %	air	52	21	0	27
	water	58	18	0	24
	land	80	0	7	13
	underground	77	0	6	17

Conclusion This technique highlighted the principal sources of environmental burdens originating from the cradle to grave of commodities supplied for the PSC processes. This method provides a transparent way to draw the system boundary for a LCI without similar previous studies or experience.

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Heavy metal uptake, bioaccumulation and histopathological responses in gills of freshwater fish from the Indus River, Pakistan

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Introduction The high nutritional value of fish makes it an ideal component of a healthy and balanced diet. Elevated levels of heavy metals in fish represent both an ecological and human health concern. Fish species are often the top consumers in aquatic ecosystems and thus metal concentrations in fish can act as an indicator of the state of the aquatic environment. Fish are known to bio-accumulate metals and are considered as one of the most indicative factors, in freshwater systems, for the estimation of trace metals pollution potential (Rashed, 2001). Histopathology can be an indicator of the effects of various anthropogenic pollutants on organisms and so could be used as a reflection of the overall health of the entire population in an ecosystem. This study aimed to assess the bioaccumulation of heavy metals in gills and muscles and their effects on histopathology of gills of *Oreochromis mossambicus* from the Indus River, Pakistan which receives potential pollutants from the domestic, municipal and agricultural runoffs.

Materials and methods Fifty-four samples of *Oreochromis mossambicus* were randomly collected from upstream (SK) and downstream (CH) sites along a stretch of the Indus River (27 samples per site) which received domestic and municipal sewage and agriculture runoffs. The fish were humanely killed, washed and dissected to obtain samples of gills and muscles. These samples were freeze dried, ground, digested in nitric acid and analysed for heavy metals by ICP-OES. The data were statistically analysed by Minitab software according to a 2x2 factorial arrangement to observe the main effects of the site and fish tissues as the main factors and their interaction on the bioaccumulation of each mineral in tissues at $P < 0.05$. Light microscopy of gill tissues was carried out by following the standard techniques with haematoxylin and eosin staining.

Results The concentrations of Mn, Pb, Hg, and Cr were higher than the acceptable limits of the World Health Organisation standards whereas the concentration of Cu was within the acceptable limits (Figure 1). Order of bioaccumulation of heavy metals in gills was $Mn > Cu > Pb > Cr > Hg$, whereas in muscles it was $Cr > Mn > Cu > Pb > Hg$. The commonest anomalies in gill histology, as shown by arrows, included dilation of the marginal channel (Figure 2a), hyperplasia of the epithelial cells and lifting of the lamellar epithelium (Figure 2b). There were some cases where the hyperplasia was more severe, resulting in the fusion of secondary lamellae (Figure 2c). Frequently, alterations such as blood congestion, hypertrophy of epithelial cells and lamellar disorganizations were also observed.

Figure 1 Bioaccumulation of heavy metals in *Oreochromis mossambicus*

Figure 2 (a,b,c) Light micrographs of fish gills inhabiting polluted water of Indus River.

Conclusion This study suggests that histopathology is useful to assess the impact of environmental stressors on fish health. The high levels of metals in the edible parts of fish from this study area could harm the health of the community of Mianwali, Pakistan. Therefore a very close monitoring of metal loads in the Indus River is recommended to reduce possible risks to the health of consumers in this area of increasing urbanisation.

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***Cyprinus carpio* as a biomarker to monitor metal pollution in the Indus River, Pakistan**

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Introduction Fish has been regarded as a source of nutritious and highly desirable food. This is due to its high quality meat which is rich in essential amino acids, beneficial fatty acids, calcium, phosphorus and vitamins. However, the pollution of the aquatic environment with heavy metals has become a serious health concern for fish and human populations. Industrial and agricultural discharges are considered as the primary source of metal poisoning of fish (Pandey, 2003). The aim of this study was to assess the bioaccumulation of trace metals in samples of skin, scales, gills and fins of fish to monitor freshwater pollutions. This information may help adopt practices to avoid the impacts of these pollutants on the aquatic and human populations.

Materials and methods This study was conducted at two sites (upstream=SK and downstream=CH) of Indus River in Mianwali, Pakistan. These sites receiving domestic and municipal sewage and agricultural runoffs were chosen to investigate the effect of various anthropogenic activities on the metal profiles of water and the most exposed tissues of Carp (*Cyprinus carpio*). This replicated 2x4 factorial experiment compared the two selected sites and four tissues (Gills, Skin, Fins and Scales) for mineral profiles of these samples. A total of 54 fish samples were collected; 27 samples of *Cyprinus carpio* from the two sampling sites. The fish were humanely killed, washed, dissected, freeze dried, ground and analysed for metal bioaccumulation using ICP-OES. Water quality was examined by following the standard methods. The data were statistically analysed by using Minitab software to observe the effect of sampling sites on water quality and the main effects of the site and fish tissues and their interaction on the bioaccumulation of each metal in fish tissues at $P < 0.05$.

Results The metals in the fish tissues (Table 1) were several folds higher than their corresponding values in the water samples from selected sites of Indus River (Table 2). Significant differences between fish tissues and sites were observed for these metals ($P < 0.05$). Variable bioaccumulation patterns of metals in different tissues were observed. The gills had the highest metal load followed by fins, skin and scales. Mn, Cu, Zn and Cr concentration in different fish tissues were higher at CH than SK site whereas, Pb concentrations were higher at SK than CH site ($P < 0.05$) (Table 1). The Indus River water was found to be suitable for aquatic life (Table 2), whereas metal levels in different exposed parts of fish except Cu were higher than the acceptable levels in food fish as reported by the World Health Organisation and Federal Environmental Protection Agency.

Table 1 Mean concentration (mg/Kg DM) of trace metals in selected tissues of *Cyprinus carpio* from two sites of the Indus River

Metal	CH				SK				Significance (P-value) for location and main effects			
	Fins	Gills	Scales	Skin	Fins	Gills	Scales	Skin	Site	Tissue	Site x tissue	
Mn	14.06	15.17	10.59	4.68	12.41	10.30	4.72	2.68	0.001	0.001	0.001	
Pb	5.76	3.84	4.31	2.08	5.41	3.74	5.51	1.83	0.048	0.001	0.054	
Cu	6.60	10.01	3.38	6.26	8.11	4.21	3.61	5.89	0.001	0.001	0.001	
Zn	253.2	1179	123	263.7	82.2	938.2	103.2	251.6	0.001	0.001	0.001	
Cr	3.43	10.76	1.74	3.47	1.88	8.54	1.56	2.55	0.033	0.001	0.02	

Table 2 Mean concentrations of trace metals in water at two selected sites of the Indus River

Metals mg/L	CH	SK	P value for CH versus SK
Cu	0.18	0.17	0.641
Mn	0.02	0.02	0.995
Zn	0.27	0.29	0.129
Cr	0.06	0.14	0.123
Pb	0.17	0.20	0.007

Conclusions The bioaccumulation patterns of different elements in different tissues can be used as an early warning indicator for water pollution. More metals in exposed parts and especially in skin which is taken as food along with muscle by the rural community, is a cause of concern as metal rich skin may harm the consumer health. The study confirmed the sub-lethal metal pollution of the Indus River where, although the fish population is surviving, the impact of heavy metal load in their tissues deserves further investigations.

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A comparison of behaviours of common squirrel monkeys (*Saimiri sciureus*) between enclosure designs at Cotswold Wildlife Park and Woburn Safari Park

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Introduction The ability of a species to adapt to their captive conditions depends on how well the enclosure resembles its natural environment (Mallapur and Choudhury, 2003). Enabling captive animals to exert some control over their environment (e.g. feeding habits) allows for high animal welfare standards which has recently been achieved through environmental 'enrichment' using complex and diverse enclosure designs (Carlstead, 1996). The principal method of assessing a captive animal's welfare is by observing their behaviours and comparing it to its wild relatives (Carlstead, 1996). Building on previous work (Williams and Litten-Brown, 2008), the objective of this study was to determine whether enclosure design has an effect on the behaviours of captive common squirrel monkeys (*Saimiri sciureus*).

Materials and methods Six monkeys (2 male and 4 female) in an enclosed exhibit at Cotswold Wildlife Park (CWP) and 14 monkeys (all female) housed in a larger walk-through enclosure at Woburn Safari Park (WSP) were observed using scan and focal sampling procedures three times a day (designed to cover feeding times) over a 5-day period (Table 1). The monkeys were individually identified by their physical characteristics and distinguishable features. A total of 17 behaviours were observed and recorded by one person throughout but only foraging, auto-grooming, huddling, aggression and climbing are presented here. Scan sampling involved recording 'snap shots' of each animal's behaviour at 10 minute intervals over a period of 1.5 hours whereas focal sampling was used to record all the behaviours performed by an individual for a 15 minute time period. The percentage time spent performing each behaviour was analysed using a two-sample t-test in Minitab[®] v15.

Table 1 A daily time plan for observing the behaviours of squirrel monkeys

	Scan Sampling	Focal Sampling
Time of day	0900-1030	1100-1115
	1130-1300	1300-1315
	1415-1545	1615-1630

Results The squirrel monkeys at CWP spent more time foraging ($P < 0.01$, Table 2) and less time auto-grooming ($P < 0.05$) than the monkeys at WSP. Although not significant there was a trend for the monkeys at WSP to spend more time huddling and showing aggression towards other individuals. Also, the monkeys at WSP were observed to be more active, showing a trend for spending more time climbing than the monkeys at CWP.

Table 2 Percentage time spent for each observed behaviour

Behaviour	Sampling method	CWP	WSP	Significance
Foraging	Scan	40.7±2.0	17.4±0.3	$P < 0.01$
Auto-grooming	Scan	3.3±0.85	6.9±0.3	$P < 0.05$
Huddling	Scan	13.0±2.0	23.6±4.1	$P = 0.081$
Aggression	Scan	0.2±0.2	1.3±0.4	$P = 0.077$
Climbing	Focal	6.7±1.5	15.0±2.9	$P = 0.062$

Conclusions The presence of zoo visitors has been characterised as both a form of enrichment and stress to captive animals (Davis *et al.*, 2005). Whilst the monkeys at WSP may benefit from the enrichment of visitors in their enclosure, they may also be exhibiting a trend for more aggression as a result of the stress (of the same visitors). The increased time spent auto-grooming could also be related to the higher levels of aggression (Kruk *et al.*, 1998), however the huddling suggests a firm interaction between the group members (Kirkpatrick *et al.*, 1997). The monkeys at CWP spent more time foraging which is in agreement with Williams and Litten-Brown (2008) who also found that foraging time was increased in an enriched environment. Whilst the authors acknowledge the limitations of 2 sites and the low number of animals available for the study, these findings indicate that both enclosures were enriched but in different forms. The results of this study suggest that the enclosure designs at CWP and WSP provided environments where squirrel monkeys could exhibit a full range of behaviours and whilst there were several differences recorded between the squirrel monkeys at CWP and WSP, neither of the enclosures appeared to be more favourable for their welfare.

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Study of resistance and vitality responses in six silkworm (*Bombyx mori* L.) hybrids under individual selection for parental lines

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Introduction Sericulture has been an important agricultural industry since 2650 BC (Seidavi *et al.* 2006). There are about 10 million farmers and 0.5 million related industrial workers in the world. The present capacity of silkworm eggs production in Iran is about 70000 boxes per year which are distributed among 40000 silkworm house holders. Annual cocoon production in Iran is about 6000 metric tons which is small in comparison with production in previous centuries. Silkworm breeding using selection systems can improve line and hybrid performance. Individual selection based on cocoon weight in GGP generation (great grandparent) improved GP (grandparent) and P (parent) parental line generation (Seidavi *et al.* 2007), but it did not consider the effects on resistance performance of hybrid generation. In this experiment, the effects of two phenotypic selections of GGP parents on vitality (percentage of survival pupae) and resistance properties in six of their hybrids (including 31×32, 32×31, 103×104, 104×103, 107×110 and 110×107) were studied.

Materials and methods In this experiment, six genetic lines, including 31, 32, 103, 104, 107 and 110, and their hybrids were studied. Silkworm rearing techniques included single batch rearing system for pure line rearing and mixed rearing system for hybrid rearing. Feeding and other conditions of larval rearing were carried out following standard procedures (ESCAP, 1993). In order to estimate resistance parameters, the data obtained from 4 rearing periods or generations were used. In 1-3 generations (GGP, GP and P), 12 families were reared so that 25 male and 25 female full sib cocoons were reared and produced next generation silkworm eggs. In the fourth generation (hybrid), four replications of 1250 larvae were reared and their resistance traits were recorded. Mating in the first and second generations were as full sib mating. But mates in third generation (i.e. P generation for hybrid silkworm egg production) were as hybridization type. For investigation on vitality responses (percentage of pupae surviving) against individual selection for parental lines, all six studied lines were divided into two groups A and B. At the first generation (GGP) all selections in population A were carried out at random. In population B, 1% superior individuals were selected as parents. Thereafter, all selections were applied in populations A and B at randomly for second (GP), third (P) and fourth (Hybrid) generations for all six lines. Six hybrids were produced from these two group lines (A and B for each line). All resistance traits were recorded and analysed for populations A and B in each hybrid. Response differences (A-B) between these two groups were calculated in each hybrid for vitality (percentage of survival pupae) and resistance traits.

Results Some resistance responses in six silkworm hybrids against individual selection for parental lines are summarised in Table 1. Results showed that the difference among hybrids for response to parents selection was variable and significant ($P<0.01$). The 31×32 hybrid showed the highest selection response (+0.25 larvae) for live larvae number. The 103×104 and 31×32 hybrids revealed the highest response for live pupae numbers (+3.00 and +0.75 larvae, respectively) and the 107×110 hybrid had the lowest response (-9.25 larvae). The maximum and minimum responses for percentage pupae of surviving (pupae vitality percentage) belonged to the 103×104 (+2.515%) and 107×110 (-0.463 larvae) hybrids, respectively. Therefore, individual selection can improve vitality (and resistance properties most in 31×32, 32×31, 103×104 and 104×103 hybrids. This is due to positive correlations between cocoon and vitality traits and heterosis phenomena.

Table 1 Resistance and vitality responses (percentage of survival pupae) in six silkworm (*Bombyx mori* L.) hybrids against individual selection for parental lines

Trait	Variety	31×32	32×31	103×104	104×103	107×110	110×107
Alive Larvae Number		-6.750 ^{ab}	0.250 ^a	-3.000 ^{ab}	-14.000 ^b	-8.500 ^{ab}	-0.500 ^a
Alive Pupae Number		-5.500 ^b	0.750 ^a	3.000 ^a	-5.750 ^b	-9.250 ^b	-1.500 ^b
Pupae Vitality Percentage (%)		0.310 ^a	0.225 ^a	2.515 ^a	3.195 ^a	-0.463 ^b	-0.433 ^b
Pupae Vitality Percentage in Best Cocoon (%)		0.145 ^a	-0.225 ^b	4.155 ^a	1.578 ^a	-0.663 ^b	-0.073 ^b
Pupae Vitality Percentage in Middle Cocoon (%)		-5.983 ^b	-4.950 ^b	5.390 ^a	3.548 ^a	0.833 ^a	1.508 ^a

For each row, means which have common letters are not different significantly ($P>0.01$)

Conclusion This experiment demonstrated that individual selection is a benefit for silkworm breeding. Thus it resurrects an old principle in animal breeding. We can convert group selection to individual selection in studied pure lines. Such an approach has the potential to greatly improve economic gains.

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Effects of dry period length on milk production and composition in early lactating Holstein cows

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Introduction Recently, there has been an interest in shortening the non-income-producing dry period (DP). Retrospective analysis of farm data and planned experiments both indicate about a 5 to 6% loss in milk yield during the subsequent lactation when the DP is reduced by approximately 30 d (Rastani *et al.*, 2005). Additional milk produced during the extra 30 d of lactation can compensate for some of the loss in the subsequent lactation. There are, however, some advantages to reducing the dry period length, including increased income from milk in the current lactation, simplified management of dry cows through movement toward a one-group nutritional strategy, decreased metabolic disorders, and reduced strain on dry cow facilities. The aim of this study was to determine the effects of dry period length on milk production and composition in early lactating Holstein cows.

Materials and methods Holstein cows (n=13) were used to evaluate 2 dry period (DP) management strategies on postpartum milk yield and composition. The cows were randomly assigned in 1 of 2 treatments: 1) traditional 60 days dry period (n=6); 2) a shortened 35 days dry period (n=7). All cows were fed by routine ration of farm (total mixed diet) twice a day at 0800 and 1400 h and had at all time free access to water. After parturition, cows were milked 3 times per day at 0400, 1200 and 1800 h and yields were recorded until 50 days postpartum. Milk samples were collected from each milking on 1 d per wk and composited for analysis of milk composition (Micro Scan; FOSS Electric A/s, Denmark). 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 Table 1 shows the effect of different dry period lengths on milk yield and composition. Milk yield, and also protein and lactose contents were all similar among the treatments. Milk fat content reduced in the shorter dry period group (P=0.02). The effect of time was significant and milk yield increased over the time (P<0.05). The milk component yields were all similar among the treatments. The effect of time was significant and the yields were increased over the time (P<0.05). Our result on milk yield is in agreement with other study showed that cows with 30 days dry period had similar milk production compare with the control (Gulay *et al.*, 2003). Result on milk fat content is in contrast to other recent study (Watters *et al.*, 2008).

Table 1 Least square means of milk yield and composition in cows with different dry period lengths.

Parameter	Dry period length, d		SE	Treatment P value	Time P Value
	60	35			
Milk, kg/d	38.34	37.96	0.734	0.72	0.02
Fat					
%	3.99	3.74	0.104	0.02	0.54
Kg/d	1.50	1.43	0.084	0.58	0.04
Protein					
%	3.05	3.15	0.037	0.09	0.29
Kg/d	1.17	1.21	0.074	0.74	0.003
Lactose					
%	4.55	4.68	0.059	0.15	0.52
Kg/d	1.75	1.79	0.111	0.77	0.007

Conclusions Results of this study demonstrated that milk yield, and also milk protein and lactose contents were not affected by shortening dry period. Based on milk yield response, a 35d dry period was sufficient time for the mammary gland to complete its renewal. More studies with larger group of cows needed to evaluate the effect of shortened dry period.

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Effect of different calving classes in the first calving on subsequent milk production in Holstein dairy cows

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Introduction Revenue from dairy farms is directly dependent upon reproductive efficiency because it affects milk production and the number of calves born (Thurmond *et al.*, 1990). Pregnancy loss can have devastating effects on economical success in dairy farms. Normal annual abortion risks have been cited to be 3–5%, once cows are beyond 42 days of pregnancy (Hovingh, 2002), or similarly an observable 2–5% in most dairies (Kirk, 2003). Although, there is little or no indication that milk production is a risk factor for increased pregnancy losses in dairy cattle (Santos *et al.*, 2004), there is little information on the effect of pregnancy losses on subsequent milk production. The aim of this study was to evaluate the impact of different calving classes including eutocia, dystocia, stillbirth and abortion in the first calving on subsequent milk production in Iranian Holstein dairy cows.

Materials and methods Data regarding to all first calving of cows were collected during 1987 until 2006 from a large commercial Holstein farm. During the period the median number of cows in the study herd was 1000. Farmer has recorded information about all existing and culled cows. Each cow has been characterized by demographic data (birth date and first calving date), production data (cumulative first 60 and 100 days milk productions as well as the 305 day milk yield), and reproduction data (calving class and sex of calf at calving). Collected data were checked for consistency of data. Finally, during the study period, a total of 2490 culled cows (females having calved at least one) were used. The dependent variables analyzed were the cumulative first 60 and 100 days milk production as well as 305 d milk yield. The model included calving class with 4 levels (including eutocia, dystocia, stillbirth and abortion), calf sex, and calving year and season. Data were analyzed by procedure GLM of SAS (2001).

Results In overall, probabilities of eutocia, dystocia, stillbirth and abortion in the first calving during the study period were 0.83, 0.09, 0.05, and 0.02, respectively. The rate of abortion in this study was in agreement with previous reports in most dairies (Kirk, 2003). Table 1 shows the effect of calving class on subsequent milk production. The milk yields increased over time ($P<0.0001$). The first cumulative 60 d milk production was affected by the calving class, and also calving year and season ($P<0.01$). Calving season impacted the 60 d milk yield and cows calved in winter produced more milk in their first 60 days of milking ($P<0.002$; 1505.3 ± 22 , 1530.6 ± 22 , 1554.6 ± 23 , and 1584.1 ± 23 , respectively for spring, summer, autumn and winter). The first cumulative 100 d milk production was also affected by calving class, calving year and season ($P<0.05$). The trends were similar to the first 60 d milk yield. Calving year and season impacted the 305 d milk yield ($P<0.001$). Cows calved in winter had the greatest milk yield in their first lactation compared with the other seasons ($P<0.001$).

Table 1 Least squares means ($\pm$ SEM) of production parameters in the first lactation of cows calved under different classes.

Parameters	Calving class				P value
	Eutocia	Dystocia	Stillbirth	Abortion	
First cumulative 60 d milk production, kg	1600.3 $\pm$ 11	1583.8 $\pm$ 23	1545.2 $\pm$ 32	1445.2 $\pm$ 55	0.008
First cumulative 100 d milk production, kg	2639.5 $\pm$ 19	2650.5 $\pm$ 38	2586.4 $\pm$ 51	2384.9 $\pm$ 87	0.02
305 d milk yield, kg	7004.7 $\pm$ 53	7114.6 $\pm$ 113	6960.1 $\pm$ 153	6998.1 $\pm$ 264	0.77

Conclusions The results of this study demonstrated that the calving classes impacted the first 60 and 100 days milk yields with no apparent effect on subsequent 305 d milk yield. Besides on the calving classes other factors impacted the first lactation production parameters. Therefore in practical terms increase incidence of abortion in herd reduces milk production in the first 100 days of lactation period.

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The effect of days dry on milk production and fertility in subsequent lactation of high producing Holstein cows

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Introduction Several studies have indicated that reducing the length of the dry period to less than 60 days had a more detrimental effect between the first and second lactation than between later lactations (Annen *et al.*, 2004). Other studies demonstrated that a dry period less than 40 days reduces milk yield in the subsequent lactation (Sørensen and Enevoldsen, 1991). Several experiments designed to examine the effects of reducing the days dry to approximately 4 weeks have shown no difference in milk production and/or fat corrected milk in the subsequent lactation (Gulay *et al.*, 2003). The aim of the present experiment was to determine the effect of days dry (from 10 to 90 days, 10 days interval) on subsequent milk production and fertility of high producing Iranian Holstein cows.

Materials and methods One hundred and ninety-two multiparous Holstein cows were selected from a herd with 750 milking cows. All cows had less than 420 days in milk at pre-partum. Animals were assigned randomly in six groups based on dry period length of 10-30 (D1030), 31-40 (D3140), 41-50 (D4150), 51-60 (D5160), 61-70 (D6170) and 70-90 (D7090) days. The experiment was carried out from September 2007 to September 2008. At assignment, parity of cows, body weight and body condition scores ranged from 2 to 9, 660 to 740 kg and 3.2 to 4.3, respectively. Cows in D4150, D5160, D6170 and D7090 were dried off and moved to a far-off (50-60 days from expected calving) dry herd with dry matter intake of 3 kg lucerne hay, 5 kg maize silage, 1.5 kg wheat straw and 3 kg far-off concentrate (CP: 162 g/kg; ME: 12.4 MJ/kg DM). The animals were moved to the close-up (20-30 days from expected calving) dry herd around 28 days before calving. Cows in D1030 and D3140 were dried off and moved directly to the close-up dry herd. The close-up dry ration (DM basis) was consisted of 2.2 kg lucerne hay, 6.1 kg maize silage, 1.5 kg wheat straw, 5.5 kg concentrate (CP: 226 g/kg; ME: 13.2 MJ/kg DM), and 400 g of anionic salts. After parturition, all cows were fed a total mixed ration (as DM) based on 25% lucerne hay, 17% maize silage and 58% concentrate. This ration met the requirements of high producing cows (CP: 182 g/kg; ME: 12.4 MJ ME/kg DM). All cows have been subjected to a presynch-ovsynch protocol around 14-24 days postpartum. During the lactation, weekly milk production was recorded. Inseminations per pregnancy and days open of all cows were recorded. Data were analyzed in a completely randomized design of GLM procedure of SAS (1999), included the treatment effect of dry period. Data of 305 d milk yield equivalent covariately adjusted for previous 305 d milk yield. Parity was used as a covariate in all statistical analysis. Tukey test was used to compare the means ($P < 0.05$).

Results Adjusted milk production (305 d equivalent), average milk production, days open and insemination per pregnancy are presented in the Table 1. Postpartum milk production was significantly lower for cows on D1030 compared with the other animals ($P < 0.05$). However, both days open and insemination per pregnancy were similar between the animals.

Table 1 The effect of days dry on milk production and fertility characteristics (Least Squares means $\pm$ SE) of the cows involved in the experiment

Item	Days dry						s.e.m	P
	10-30	31-40	41-50	51-60	61-70	70-90		
No of cows	13	11	37	69	38	24		
Adjusted 305 d milk yield (ton)	8.3 $\pm$ 0.4 ^a	9.4 $\pm$ 0.5 ^{ab}	9.9 $\pm$ 0.3 ^b	10.4 $\pm$ 0.2 ^b	10.1 $\pm$ 0.3 ^b	10.7 $\pm$ 0.4 ^b	0.42	0.01
Average milk (kg/d/head)	28 $\pm$ 1.9 ^a	33 $\pm$ 2.1 ^{ab}	34 $\pm$ 1.1 ^{ab}	35 $\pm$ 0.9 ^b	36 $\pm$ 1.2 ^b	38 $\pm$ 1.6 ^b	1.7	0.05
Days open	183 $\pm$ 25	131 $\pm$ 27	188 $\pm$ 15	182 $\pm$ 11	171 $\pm$ 14	186 $\pm$ 18	32.8	0.55
Insemination/pregnancy	3.7 $\pm$ 0.6	3.4 $\pm$ 0.7	4.1 $\pm$ 0.4	3.8 $\pm$ 0.3	3.8 $\pm$ 0.4	4.1 $\pm$ 0.6	0.9	0.76

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

Conclusions Results of the current study showed evidence that shortening the dry length to 30 days caused a negative effect on subsequent milk production. There is sufficient evidence to question if the most appropriate dry period length for a modern Holstein dairy cow is 6 to 8 weeks. It was suggested that 30 days dry may be sufficient (Gulay *et al.*, 2003). Bachman and Schairer (2003) summarized the results from several experiments in which cows were managed for varying dry period lengths. Results of the present study indicated that days dry had an effect on both adjusted 305 day mature equivalent and average milk production. However, no significant effect of days dry was observed on days open and insemination per pregnancy.

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Effects of dry period length on follicular dynamics in early lactating Holstein cows

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Introduction For profitable production of milk, a nonlactating or dry period is established between lactations of dairy cow. There has been substantial recent interest in shortening dry periods. Physiological studies show that the changes the udder goes through to prepare for the next lactation take about three weeks, and yet current advice is that the most appropriate dry period for a modern dairy cow is 45 to 60 days. More recent studies show good reason to question that advice, and suggest dry periods of 30 to 35 days have no detrimental effect on production, but information relating the effects of dry period to subsequent reproductive performance of lactating cows is sparse (Grummer, 2007). Also, the effect of this management change on follicular dynamic has not been adequately evaluated. Therefore, the objective of the present study was to determine the effects of dry period length on follicular dynamics in early lactating Holstein cows.

Materials and methods Holstein cows (n = 24) were randomly assigned in 1 of 2 treatments: 1) traditional 60 d dry period and 2) a shortened 35 d dry period. Holstein cows were blocked by parity (2nd and 3rd to 5th), their previous 305-day milk yield and expected calving dates. All cows were fed by routine ration of farm (total mixed diet) twice a day at 0800 and 1400 h and had at all time free access to water. To monitor follicular parameters, ultrasound measurements of follicular activity were made on alternate days from day 10 to 35 postpartum to determine the characteristics and fate of the 1st follicular wave, using a 7.5 MHz rectal transducer (Medison SA 600V, Seoul, Korea). Dominant follicle development was characterized by follicular mapping of recorded ultrasound images (Heravi Moussavi *et al.*, 2007). Follicular recruitment during the 1st follicular wave after parturition was evaluated by quantification of the numbers of 5-10 mm follicles on day 10 and 14. 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 Dry period length 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 d 10 and 14, diameter of the largest follicle on d 10 (at first ultrasound), diameter of the first dominant follicle on d 14, maximum diameter of the first dominant follicle, number of days until detection of a follicle ≥ 10 mm in diameter, and days to first ovulatory follicle were all similar among the treatments. Our results are in contrast to Gumen *et al.* (2005) study that showed follicle diameter at first ultrasonography, days to detection of first 10-mm follicle, and days to first postpartum ovulation were impacted by dry period length.

Table 1 Ovarian follicles and development during the first follicular wave postpartum in cows with 60 and 35 d dry periods.

Parameter	Dry period lengths, d			
	60	35	SE	P
Number of follicles (5-10 mm in diameter) on day 10	4.25	3.83	0.570	0.61
Number of follicles (5-10 mm in diameter) on day 14	5.00	5.10	0.631	0.92
Diameter of the largest follicle at first ultrasound, mm	10.66	10.54	0.843	0.91
Diameter of first dominant follicle on d 14, mm	13.37	12.95	0.844	0.73
Diameter of the first dominant follicle, mm	19.41	19.58	1.284	0.92
Number of days until detection of a follicle ≥ 10 mm in diameter	11.58	11.66	0.650	0.92
Days postpartum to first ovulation, d	27.66	27.08	2.776	0.88

Conclusions The results of this study demonstrated that the dry period lengths had no apparent effect on follicular dynamic.

Further studies are needed to evaluate the impact of shortening dry period on follicular dynamic and reproduction parameters.

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On-line application of visible and near infrared reflectance spectroscopy to predict physical and sensory characteristics of beef quality

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Introduction Visible and near infrared reflectance spectroscopy (Vis-NIR) has been widely used by the industry research-base for large-scale meat quality evaluation to predict the chemical composition of meat quickly and accurately. Meat tenderness is measured by means of slow and destructive methods (e.g. Warner-Bratzler shear force). Similarly, sensory analysis, using trained panellists, requires large meat samples and is a complex, expensive and time-consuming technique. Nevertheless, these characteristics are important criteria that affect consumers' evaluation of beef quality. Vis-NIR technique provides information about the molecular bonds (chemical constituents) and tissue ultra-structure in a scanned sample and thus can indirectly predict physical or sensory parameters of meat samples. Applications of Vis-NIR spectroscopy in an abattoir for prediction of physical and sensory characteristics have been less developed than in other fields. Therefore, the aim of this study was to test the on-line Vis-NIR spectroscopy for the prediction of beef quality characteristics such as colour, instrumental texture, water holding capacity (WHC) and sensory traits, by direct application of a fibre-optic probe to the *M. longissimus thoracis* with no prior sample treatment.

Materials and methods Cross-bred steers and heifers (n = 194; sired by either Aberdeen Angus or Limousin bulls) were used in this three-year study. The animals were slaughtered in 11 batches from autumn 2006 until spring 2008 at an average carcass weight and age at slaughter of 335 (SD = 30.3) kg and 584 (SD = 26.2) days, respectively. Vis-NIR measurements were taken from the left side carcass *M. longissimus thoracis* (13th rib region) at 48 h *post mortem* (pm), by means of a NIR spectrophotometer (Qualityspec Pro, ASD Inc., Boulders, Colorado) provided with a sampling fibre-optic probe. Meat samples were scanned at every 1 nm over the Vis-NIR spectral range (350 to 1,800 nm). The absorbance data, stored as log (1/R), were subjected to multiplicative scatter correction and/or derivatives. Calibrations were performed using partial least squares regression. Full cross-validation was applied and the best equation was selected on the basis of minimising the standard error of cross-validation (SE_{CV}). Meat colour (CIE L*a*b*) was measured at the same position and time as Vis-NIR measurements. Sensory characteristics (texture, juiciness, beef flavour, abnormal flavour and overall liking) were assessed on 1-8 scale by a trained taste panel on meat samples (joint section including 11-12th ribs) aged for 14 days. The *M. longissimus lumborum* was used to determine the WHC and the instrumental texture. Meat aged for 14 days was weighed, cooked in a clam shell grill to a tissue centre temperature of 71 °C and reweighed to calculate cooking loss (WHC). The slice shear force (SSF) test was performed on hot cooked meat (at 3 and 14 days pm) according to Shackelford *et al.* (1999); a single meat sample of 50 mm by 10 mm was cut orthogonal to muscle fibre orientation. Samples of 10 days aged meat were cooked in a water bath until a tissue centre temperature of 78 °C and 10 replicate blocks (20 x 10 x 10 mm) were cut parallel to the fibre direction and sheared across the fibres with a Volodkevitch jaw.

Results Vis-NIR calibrations, tested by cross-validation, showed high predictability for L*, a* and b* (R² = 0.863, 0.861 and 0.907; SE_{CV} = 0.965, 0.949 and 0.692; respectively; Table 1). The accuracy of Vis-NIR to estimate WHC and instrumental texture ranged from R² = 0.31 to 0.54, suggesting moderate prediction ability for these traits. The Vis-NIR equations to predict sensory characteristics resulted in R² in the range from 0.21 (juiciness) to 0.59 (flavour). The correlations of Vis-NIR measurements and several meat quality traits ranged from 0.46 to 0.95.

Table 1 Ranges, means, standard deviations and prediction statistics of physical and sensory characteristics of beef samples

	Range	Mean	SD	R ²	SE _{CV}
L*/a*/b* colour	31-54/17-29/3-15	38/24/8	2.8/2.1/1.8	0.86/0.86/0.91	0.96/0.95/0.69
Volod./SSF3/SSF14 (N)	17-98/89-386/61-273	48/193/125	13.9/69.8/34.6	0.37/0.54/0.31	12.70/59.45/28.49
Cooking loss (%)	16-30	24	2.7	0.35	2.35
Texture/Juiciness	3.0-6.7/3.8-5.9	4.8/4.8	0.70/0.43	0.28/0.21	0.60/0.41
Flavour/Abnormal flav.	2.6-5.7/2.0-4.0	4.3/2.9	0.56/0.41	0.59/0.22	0.42/0.37
Overall liking	3.1-5.7	4.3	0.44	0.25	0.38

Conclusions The results of this research support the use of on-line Vis-NIR spectroscopy for the estimation of beef colour, a parameter closely associated with freshness, ripeness or desirability, factors which are often a primary consideration for consumers when making purchasing decisions. Considering that the Vis-NIR measurements were taken at different locations and at an earlier time *post mortem* compared to those used for sensory characteristics, WHC and instrumental texture, the correlations obtained indicate that Vis-NIR could be used in the abattoir as an early predictor for meat eating quality on-line. Under practical conditions, much more variation in meat quality is expected than for these experimental animals and so these correlations may be at the lower end of those possible for predicting meat eating quality.

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Organoleptic properties of seasoned and unseasoned dry meat products from beef and camel meat

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Introduction The dearth of animal protein in the diets of persons living in developing countries has been an issue of concern to Governments and individuals over the years. This is because what is obtained from the major sources of meat apply can no longer sustain the growing demand due to increases in human population (Vietmeyer, 1985). In order to bridge the gap between demand and supply of animal protein, preservations of meat is therefore very necessary. 'Kundi' is a dry meat product traditionally produced in the northern part of Nigeria. It is conventionally produced unseasoned from camel meat. Therefore the aim of this study is to improve on the traditional method, to increase the organoleptic properties and to produce 'Kundi' from other animal like beef.

Materials and methods Semimembranous muscles weighing 6 kg from 2 to 3 years old male *Camelus dromedarius* and White Fulani animal were used. The muscles were trimmed off of all external blood vessel, nerves, fat, excess epimysial connective tissue and bones. The chunks were held overnight for 24 hours at 4 °C and cut into sizeable smaller portion within the range of 70 – 90 grammes of 6 cm to 8 cm wide. Each meat were boiled for 20 minutes at 100 °C, during boiling, ingredients like onion, thyme, curry powder, monosodium glutamate and common salts were used to seasoned before drying. Boiled meat samples were oven dried at 150°C – 175°C for 3 hours and allowed to equilibrate to room temperature.

A total number of twenty trained individuals were used. The panellists were male (n-9) and female (n-11) and ranged in age from 27 to 35 years. The panellists were randomly allocated to the four treatments. The panellists were made to rate each of the three replicate of meat samples. Equal bite size from each treatment were coded and served in an odourless plastic plate. Each sample was evaluated independent of the other. The panellists rated the samples on a nine point hedonic scale for tenderness, flavour, colour, juiciness, texture and overall acceptability.

All data obtained were subjected to analysis of variance (ANOVA), and where statistical significance was observed, the means were compared using the Duncan's Multiple Range (DMR) test. The SAS computer package was used for all statistical analysis (SAS, 1999).

Results Significant differences were observed in all the treatments observed. Dried meat products ('Kundi') from seasoned beef had the highest significant organoleptic properties than seasoned camel products and unseasoned products from beef and camel meat. The unseasoned products from camel meat had the lowest texture 3.10 compared to 6.30, 3.60 and 5.90 obtained in seasoned beef, camel and unseasoned beef respectively, while overall acceptability was highest 7.00 for seasoned beef products than 5.90 seasoned camel, 4.62 unseasoned beef, and 4.50 unseasoned camel ('Kundi') products.

Table 1 Organoleptic evaluation of seasoned and unseasoned 'kundi'

Parameter	Treatments				SEM
	Seasoned		Unseasoned		
	Beef	Camel	Beef	Camel	
Tenderness	6.50 ^a	5.70 ^b	6.23 ^a	5.57 ^b	0.23
Flavour	6.30 ^a	5.90 ^b	3.80 ^c	3.40 ^c	0.56
Colour	6.50 ^a	3.90 ^b	6.30 ^a	3.60 ^b	0.28
Juiciness	6.53 ^a	6.40 ^{ab}	4.81 ^b	4.70 ^b	0.40
Texture	6.30 ^a	3.60 ^c	5.90 ^b	3.10 ^d	0.17
Overall Acceptability	7.00 ^a	5.90 ^b	4.62 ^c	4.50 ^c	0.29

^{abc} means on the same row with different superscript are significantly different (P<0.05).

No of animal - 2

Seasoned Beef are dried beef product with the addition of ingredient

Unseasoned Beef are dried beef product without ingredient

Conclusion 'Kundi', dry meat product has high organoleptic values when seasoned especially if produced from beef. 'Kundi' could also be produced from other domestic animals like goat, rabbit and chicken.

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Finishing diets used in beef and lamb production in Northern Ireland: results of a questionnaire survey

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Introduction Ruminant feeding regimes that include grass finishing are known to increase the n-3 polyunsaturated fatty acid (n-3 PUFA) content of beef and lamb. By contrast, concentrate finishing produces meat with increased n-6 PUFA concentrations. There is strong evidence that increasing the ratio of n-3: n-6 PUFA in the diet has beneficial effects for human health. In Northern Ireland, it is likely that feeding regimes are predominantly grass- based; therefore beef and lamb could contain appreciable amounts of n-3 PUFA. However, an analysis of types of finishing diets used by farms in Northern Ireland has not been done. The aim of this study was to assess the frequency of grass and concentrate finishing in farms in Northern Ireland.

Materials and methods Using the 2006 Agricultural Census for Northern Ireland, 600 beef cattle and/or lamb- producing farms were selected using a random stratified sampling tool, ensuring that farms were equally distributed by county. The questionnaire was designed with objectives of: i) identifying finishers of beef and of lamb, ii) common times of year for animals to be finished and iii) diets used in the month prior to animals being slaughtered. There were two sections; section 1 requested detail on beef production, section 2 requested details on lamb production and participants answered either section, or both as appropriate. Farmers were asked if they finish cattle or lambs in question 1. Question 2 asked farmers to identify the approximate number of animals finished and sent to slaughter by month, based on 2006 as an example of a typical year to allow easy recall. In question 3, farmers were asked to identify the main finishing diet provided in the last month prior to slaughter. One month pre- slaughter was sufficient to represent the finishing diet; due to evidence from Noci *et al.* (2005) that 40 days on grass or concentrate- based diets is enough for changes to be made to fatty acid composition of beef. The options for finishing diets given were: grass only, grass with concentrate, concentrate only, silage with concentrate and other. The questionnaire was posted in April 2007, with a stamp- addressed envelope enclosed. Non-responses were followed up by telephone call.

Results Of the returned questionnaires (16%, n=98), 34 reported finishing beef cattle, 29 reported finishing lambs and 35 reported finishing both. Taking the year as a whole, the most common finishing diet of lambs was grass (73% of farms) and of cattle, silage with concentrate (72% of farms). It was common for the majority of cattle (48%) and lambs (66%) slaughtered in summer months to be grass- finished (Table 1). Higher numbers of farms reported finishing cattle in February (49%; mean cattle n= 9 ± 1.7) and lambs in July and August (60%; mean lambs n= 33 ± 4.5 and 38 ± 5.3 respectively). When the diet provided to these animals was examined, it was found that the major diet of lambs finished in July and August was grass (68%) and of cattle finished in February was silage plus concentrate (86%). Lambs tended to be grass finished over a longer period of the year than cattle and were predominantly grass finished in autumn (55%) as well as summer months.

Table 1 Finishing diets of cattle and lambs by season

Finishing diets	Cattle				Lambs			
	Spring ¹	Summer ²	Autumn ³	Winter ⁴	Spring ¹	Summer ²	Autumn ³	Winter ⁴
Grass	8	48	16	0	28	66	55	25
Grass/ concentrate	26	39	50	16	54	32	41	53
Concentrate	4	6	2	3	8	0	0	3
Silage/ concentrate	60	7	32	81	5	0	2	16
Other	2	0	0	0	5	2	2	3

Expressed as % total cattle farm responses (n= 72) or % total lamb farm responses (n= 60)

Based on diets provided to animals finished in months ¹March, April, May; ²June, July, August; ³September, October, November; ⁴December, January, February

Conclusions Results of this survey suggest that there is substantial grass finishing of both cattle and lambs in Northern Ireland, particularly in the summer months. Such meat is likely to contain appreciable amounts of n-3 PUFA and, as red meat is consumed by 88% of the Irish population, as shown by Cosgrove *et al.* (2004), it may contribute significantly to dietary intakes of n-3 PUFA among Irish consumers. However, as it is not feasible for animals to be grass- fed over the whole year, there is likely to be seasonal variation in amounts of n-3 PUFA found within meat owing to the seasonal variation in use of diets seen in this study.

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Evaluation of period of fasting and mixing of steers prior to slaughter on meat eating quality

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Introduction Mixing of cattle prior to slaughter which results in aggressive activity (Mohan Raj *et al* 1992) leads to glycogen depletion pre-slaughter and subsequently meat with a higher ultimate pH (pHu). Purchas *et al* (1990) reported a quadratic relationship between pHu and tenderness with highest shear force values recorded between pHu 5.8 to 6.2. The aim of this study was to determine the effect of fasting and mixing of steers prior to slaughter on the meat eating quality of longissimus dorsi (LD) muscle.

Materials and methods Thirty two Holstein steers, mean initial live weight 462 (SD 40.0) kg, were penned indoors on slats in groups of 4 and offered grass silage supplemented with 4 kg concentrate for a period of 100 days. Feed was withdrawn from 2 groups at 72 hour prior to slaughter and a further 2 groups at 48, 24 and 0 hour prior to slaughter. Animals had access to water at all times. On the morning of slaughter 2 steers from each group were removed from their pen, mixed together in an open yard for a period of 1 hour and loaded onto a lorry. The remaining 2 steers in the group were removed from their pen and loaded onto the lorry immediately prior to transport. All animals were transported on the same lorry and arrived at the abattoir within 1 hour from leaving the farm. On arrival at the abattoir, animals were allocated a kill number, clipped and then slaughtered. Carcasses were hung tenderstretch and followed standard commercial chilling regimes. Forty eight hour post-slaughter the carcasses were boned out. The LD muscle was removed from the sirloin and trimmed free of fat. Slices 25 mm thick were cut from the middle third (LD_M) and posterior third (LD_P) and prepared for sensory analysis as pieces 70mm square, vac packed (5 pieces/pack) and aged until 7 days (LD_M) or 21 days (LD_P) days post slaughter. After aging they were blast frozen and stored at -21°C until required for consumer evaluation. The steaks were cooked on clam shell grill to a well done degree. The steaks were assessed by a consumer panel using a line scale for tenderness, juiciness, flavour liking, overall liking and their satisfaction rated using a 4 category scale. Each steak was evaluated by 10 consumers and the average used for statistical analysis, a total of 120 individual consumers were involved in the trial. The data was analysed using a factorial design (2 mixing by 4 fasting periods) using REML.

Results The mean live weight at slaughter was 586 kg, carcass weight 286.5 kg. There was no statistically significant effect of either mixing or fasting on pHu values. There were 2 carcasses with LD pHu values >5.8 (5.83 0 h, not mixed; 5.87 fasted 48 h, mixed). The juiciness of the LD_P (21 days aged) was significantly lower ($p < 0.05$) in the mixed group than the unmixed group (Table 1). There was no statistically significant effect of mixing or fasting on other attributes of eating quality of LD_P. There were no statistically significant effects on the eating quality of the LD_M. There were no statistically significant mixing by fasting interactions for pHu and eating quality of either the LD_M or LD_P.

Table 1 Effect of mixing and fasting of steers on pHu and eating quality of LD_P 21 days post slaughter.

	pHu	Tenderness ^a	Juiciness ^a	Flavour Liking ^a	Overall Liking ^a	Satisfaction ^b
Mixing						
Mixed	5.65	56.4	56.3	61.0	60.4	3.5
Not mixed	5.65	58.7	61.4	61.8	61.0	3.5
sed	0.076	3.42	2.62	2.84	2.63	0.13
sig	NS	NS	*	NS	NS	NS
Fasting						
0	5.63	57.9	61.5	60.7	60.9	3.4
24	5.55	61.8	60.7	65.4	64.4	3.7
48	5.69	56.9	58.9	61.0	60.1	3.5
72	5.65	53.5	54.9	58.6	57.5	3.4
sed	0.076	4.83	3.71	4.02	3.72	0.18
sig	NS	NS	NS	NS	NS	NS

^a scored on scale of: 0 to 100, with 0 = extremely tough, 100 = extremely tender etc;

^b category scale: 2=unsatisfactory, 3=satisfactory everyday quality, 4=better than everyday quality, 5=premium quality

Conclusions Fasting steers up to 72 hours prior to slaughter had no effect on meat quality. Mixing of steers for 1 hour prior to transport, when coupled with short journey and lairage duration, decreased juiciness in the LD_P, but had no other effect on eating quality in the LD.

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Predicting the slaughter characteristics of finished beef cattle using a live animal digital image analysis system under typical on-farm management conditions

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Introduction Previous work has suggested that dimensional data obtained from digital image analysis (DIA) has potential to predict the slaughter liveweights of finished beef cattle (Hyslop *et al*, 2008). Both farmers and cattle buyers may benefit from being able to select animals to meet slaughter specifications more accurately on-farm. The objective of the current study was to assess the feasibility of an on-farm DIA system to predict a number of characteristics in beef steers at slaughter.

Materials and methods Top, side and rear view images of finished beef steers were obtained within one day of the animals going for slaughter from two pens, each containing twelve Aberdeen Angus x Limousin (AAxLIM) and twelve Limousin x Aberdeen Angus (LIMxAA) finishing beef steers during late summer/early autumn 2007 where slaughter characteristics were also determined. Continuous images were taken using three multiplexed, high-resolution, colour digital cameras mounted directly above the animal looking straight down (top view), to the side of the animal looking straight-on (side view) and above and to the rear of the animal looking at a 45° angle (rear view). The cameras were positioned in a “cubicle” leading to the water trough in the corner of each 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. Dimensions collected for the body of each of the 48 animals included:- lengths, widths, areas and depths. Derived body volumes and area ratios were also calculated for each animal and examined as potential predictor variables. Simple and step-wise multiple regression (SMRL), partial least squares (PLS) analysis and artificial neural network (NN) relationships between a total of ninety-eight potential DIA predictor variables and steer slaughter characteristics were examined using Genstat 8, SAS and Neuframe analysis software.

Results No major problems were encountered with practical operation of the DIA system in an on-farm environment. The adjusted R^2 and residual standard deviation (R.S.D) values for the optimum regression relationships between DIA predictors and slaughter characteristics (liveweight {LW}, cold carcass weight {CCW}, killing out proportion {KO prop}, sirloin weight, sirloin proportion {sirloin prop}, fat and conformation gradings on the EUROP grid scale) are given in Table 1. The range in actual data values to be predicted were as follows: LW (534-680), CCW (287-391), KO prop (521-613), sirloin weight (22.7-34.5), sirloin prop (67.6 – 100.1), fat grade (3, 4L & 4H), conf grade (R & -U). Significant ($P < 0.01$) predictive relationships were established for all slaughter characteristics and the RSD figures represent an ability to predict the actual slaughter value with an error of +/- 1.0 - 7.9%. Although on a limited dataset of 2 or 3 categorical EUROP gradings, NN analysis was also able to correctly predict fat and conformation gradings in 47 of the 48 steers. The predicted vs actual slaughter LWs using an optimum multiple regression relationship are shown in Figure 1.

Table 1 Adjusted R^2 and R.S.D of optimum predictive relationships between DIA dimensions and slaughter characteristics of finished beef steers.

Slaughter characteristic		R^2	R.S.D.
LW	(kg)	0.807	15.7
CCW	(kg)	0.813	10.4
KO prop	(g/kg)	0.909	5.3
Sirloin weight	(kg)	0.584	2.1
Sirloin prop	(g/kg)	0.613	5.1
Fat grading (EUROP)		0.806	
(PLS analysis)			
Conf grading (EUROP)		0.807	
(PLS analysis)

Figure 1 Predicted vs actual slaughter LWs using an optimum multiple regression model from DIA predictors ($R^2 = 0.807$; R.S.D. = 15.7).

Conclusions This study has established that live animal DIA systems are feasible under common on-farm management conditions and can be used to predict some important slaughter characteristics. Further studies are required to extend the DIA database to cover additional animal groups (e.g. bulls and heifers, different breeds) and more divergent fat and conformation grading combinations. More robust prediction equations could then be developed to cover this wider range of commercial cattle. The potential of live animal DIA systems in an auction market environment should also be examined.

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Effects of supplemental vitamin E and packaging system on the shelf life of venison from red deer (*Cervus elaphus*) finished off grass or concentrates

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Introduction On exposure to oxygen, venison discolours at a faster rate than lamb, beef or pork (Trout and Gutzke, 1995) and a short shelf life is a problem for meat retailers. Increasing antioxidants in meat through the diet, and the type of packaging system used, can substantially improve shelf life. This study determined the effects of supplemental vitamin E and packaging system on colour stability and fat rancidity in venison from red deer finished off grass or concentrates.

Materials and methods A preliminary trial compared colour (chroma) and lipid peroxidation (as thiobarbituric acid reactive substances, TBARS) of venison in three pack types. Eleven stags, finished at ADAS Rosemaund on a standard grass-based system, were slaughtered at 18 months of age. Loin (*longissimus lumborum*) and topside (*semimembranosus*) muscles were aged for five days under vacuum, cut into steaks, packed in a modified atmosphere (MA: O₂:CO₂, 80:20), overwrapped (OW) or vacuum packed (VAC) and subjected to simulated retail display (16h light, 8h dark, 3°C, 700lux). Colour was measured on the surface of each steak through the pack film with a Minolta-Chromameter CR400 (Minolta Camera Company, Milton Keynes), daily for OW and MA packs and every 3rd and 7th day for VAC packs. TBARS were measured on days 4 and 7 of display (MA and OW) or days 14 and 35 (VAC). A feeding trial, lasting on average 109 days, used four groups of ten 22-month old hinds in a 2 x 2 factorial design, involving two feed treatments, with and without vitamin E (fed as 4 g/head/day ROVIMIX E-50 adsorbate to provide 2g vitamin E). The feed treatments were grazed ryegrass/clover swards and indoor-fed baled silage plus 1.0kg/head/day of concentrates (whole barley:soya, 90:10). Chroma and TBARS were measured in MA and VAC packed loin samples. In addition, muscle vitamin E was extracted, separated and quantified by HPLC. An ANOVA with diet and days as factors was used and means compared post hoc by the Tukey-Kramer test. For chroma and TBARS, day was nested within animal.

Results In the preliminary trial, chroma reduction was delayed by approximately two days in MA compared with OW and the rate of deterioration was faster in the 'redder' topside than in the loin (Fig. 1)

In VAC, chroma did not decline until day 17 after which the rate of deterioration was again faster in the topside. At 4 days the only significant difference in TBARS was between OW (2.2) and MA topside (3.8, $p < 0.05$) but all values were > 2.0 , indicating the development of rancidity, even at this early stage. By 7 days, all values were high although OW was significantly ($p < 0.001$) lower than MA loin and MA topside (5.9, 15.1 and 13.5, respectively). In the feeding trial, supplementary vitamin E in the diet significantly increased muscle vitamin E with the bigger effect in the concentrate fed group (sed = 0.31, Table 1) and in both grass and concentrate diets it prolonged shelf life, by about two (grass) or three (concentrates) days (Fig. 2). The TBARS in the meat from the MA packs reflected these antioxidant effects with significantly lower values in the groups fed the supplemented diet (max. sed = 1.18, Table 1), but there were no corresponding effects in the oxygen deprived packs (VAC) where little oxidation occurred.

Table 1 TBARS and vitamin E (mg/kg meat)

	TBARS				Vit E
	MA		VAC		
	4d	7d	14d	35d	
Conc	9.7 ^a	16.2 ^c	0.2 ^b	0.2 ^b	2.1 ^a
Conc +E	1.2 ^b	3.1 ^b	0.2 ^b	0.2 ^b	5.6 ^c
Grass	8.1 ^a	17.2 ^c	0.2 ^b	0.2 ^b	4.6 ^b
Grass+E	2.5 ^b	9.4 ^a	0.2 ^b	0.2 ^b	5.9 ^c

a, b, c means (within TBARS/Vit E) with different superscripts are significantly different ($p < 0.05$)

Conclusions MA and vitamin E feeding prolonged the colour shelf life of venison but samples in MA had undergone extensive lipid peroxidation by day 7, even with vitamin E. Vacuum packing for retail display may be the best method for this meat.

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Evaluation of measurements that may define venison quality parameters in UK farmed deer

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Introduction Early work established that carcasses of deer, compared with other species, were leaner, yielded a greater proportion of 'first class meat', that venison had a beneficial fatty acid composition and was dark in colour (Blaxter *et al.*, 1974). More recently, Fisher *et al.* (1998) and Stevenson-Barry (2000) have reviewed and identified potential important 'quality' parameters for venison. From these, the UK deer industry needs to identify a suite of measurements to define 'quality', to improve on the current subjective assessments of carcasses in commercial abattoirs and to help guide future production systems. The aim of this study was to identify suitable quality measurements to characterize UK venison.

Materials and methods Six deer slaughter premises, covering the full range of slaughter systems and kill throughputs, were used to sample 577 deer, slaughtered throughout the year at ages 15-27 months, reflecting the animal/seasonal variation of UK deer production systems. Measurements of temperament, muscle pH, colour, shelf life, texture and fatty acids (FA) were made. Trained operators assessed temperament just prior to slaughter on a scale 1-4 (1=placid, 4=agitated). The ultimate pH (pH_u) of the *longissimus lumborum* muscle (LL) was measured 24h after slaughter using a Testo 205 probe. Colour of LL was assessed, after blooming for one hour, by trained operators matching it to one of seven shades on a colour card developed specifically for venison. LL samples were removed at 24h from a sub-set of 80 carcasses, aged for 5 days under vacuum and cut into steaks. These were stored at 3°C under lights (16h light, 8h dark, 700lux) and colour was measured daily through the pack film with a Minolta CR400 Chromameter. For texture measurement, regular size samples of thawed LL were cooked in a circulating water bath at 80°C until the internal temperature reached 78°C and cooled rapidly. Samples were cut into 1x1x2cm blocks 24h later and the shear force measured using Volodkovitch jaws mounted in a Stevens CR Analyser. Another thawed and trimmed LL sample was homogenized and fatty acids extracted by direct saponification, methylated and analysed by gas chromatography. An ANOVA was used to assess the significance of main factor effects for continuous data, with Kruskal-Wallis and median tests used for score data.

Results

Table 1 Means and SE of means for the different attributes measured

	N	Mean	SE		N	Mean	SE
Temperament (scale 1-4)	577	1.53	0.031	Shelf life (chroma, day 1)	75	16.7	0.281
pH	567	5.53	0.005	Texture (kgf)	78	5.07	0.292
Colour (scale 1-7)	528	3.60	0.051	Total FA (mg/100g muscle)	80	1209	34.5

Of the 577 deer, 92% were 'placid' or 'alert' pre-slaughter and only 3% were 'agitated' (mainly 21-month males). Overall, temperament deteriorated with increasing age in males ($P < 0.001$), but not in females, and in abattoir-killed deer compared with field-shot animals ($P < 0.001$). Muscle pH_u was consistent across sexes and age groups, but two deer had pH_u > 5.8 and had high shear force values (>8.0 kgf). Colour assessed by shade card showed field-shot deer consistently produced darker muscle than abattoir-killed deer ($P < 0.001$). Muscle chroma values decreased from 19.8 ± 0.34 at 1h post-cutting to 12.0 ± 0.23 on day 5, but there were only small differences due to age and gender. Muscle shear force values demonstrated wide variation and 0.17 and 0.13 proportions were 'very tender' (<3.0 kgf) and 'very tough' (>8.0 kgf), respectively. Shear force was higher for males than females of equivalent age (Fig. 1, $P < 0.05$) and there was a tendency for tougher samples to be derived from deer having higher temperament scores at slaughter ($P = 0.056$). Venison muscle had a very low fatty acid content (total FA 1209 ± 34.5 mg/100g); only 0.33 and 0.25 of that found in beef sirloin steaks and lamb chops, respectively (Enser *et al.*, 1996). Regardless of age, total FA content was consistent at 0.11-0.13 (Fig 2), and there were no differences between males and females or in the ratio of polyunsaturates to saturates (P:S ratio), which was high at 0.66 ± 0.033 .

Figure 1 Mean shear force value (kgf) by age and gender

Figure 2 Sum weight of fatty acids by age and gender

Conclusions This study quantified some of the parameters defining venison quality and indicated that there are associations between some pre- and post-slaughter measurements. The large variation in muscle texture needs further investigation.

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Evaluation of techniques to predict the percentage lean meat of primal cuts from pig carcasses

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Introduction Each primal cut of a pig carcass has a separate market and value. Commonly the degree of blemishes on the skin of the cut is the main determinant of its quality. However, the lean meat percentage of the primal cut is also of importance, especially to the consumer. If prediction equations were in place to enable processors to more accurately estimate the lean meat percentage of specific primal cuts, it would allow optimisation of primal cuts for specific markets or processing operations. The experiment investigated the accuracy of lean meat prediction equations for primal cuts using indicator cuts, grading probe data, data from photographic images and whole carcass lean meat percentage values.

Materials and methods A full description of the materials and methods used in the current experiment are described by Magowan *et al.* (2009). In summary, 161 pigs (Landrace x Large White) representing a range of carcass weights and back fat depths were used. The back fat depth of hot carcasses was measured at P₂ (65mm from the midline at the level of the last rib) using the Ulster, Hennessy and Optical grading probes. After dissection (Walstra and Merkus, 1996), the lean meat percentage (LM%) of each primal cut (loin, shoulder, leg and belly) was determined. In addition the loin was cut into 4 sections: between the 9th and 10th rib, just after the last rib and again a further 10cm towards the rump end of the loin. This 10cm segment was termed 'Loin C'. These cuts exposed 5 cross sectional areas (A (shoulder end) to E (rump end)). Photographs of the 5 cross sections were taken at a fixed distance and magnitude. Using SigmaScan Pro, the LM% of the cross sectional photograph was calculated. Regression analysis using Genstat (Version 11) was used to statistically analyse the results. The data set was randomly split with 2/3 of data being used to establish the equation and the remaining 1/3 validated the equation. An accurate equation was identified as one with high R² (> 0.65) and low RMSEP values (<= 2.5).

Results The lean meat percentage of each primal cut was most accurately estimated using the lean meat percentage of the whole carcass (Table 1). Prediction equations for lean meat percentage of the loin, leg and shoulder using P₂ values measured by the Ulster and Hennessy grading probes were also accurate. In predicting lean meat percentage of the belly, the only predictor that could be considered as producing an accurate equation was the lean meat percentage of the whole carcass. The use of lean meat percentage determined from the photographic images of the cross sectional areas of the loin were more accurate at predicting the lean meat percentage of the primal cuts than the P₂ values and 'Loin C'.

Table 1 The accuracy of LM% prediction equations for primal cuts

Dependant:	Predictor:	R ²	RMSEP	Dependant	Predictor:	R ²	RMSEP
Loin LM%	LM% (carcass)	0.82	2.34	Shoulder LM%	LM% (carcass)	0.87	1.14
	Loin C LM%	0.87	2.42		Loin C LM%	0.61	2.18
	Ulster Probe P ₂	0.76	2.36		Ulster Probe P ₂	0.58	2.14
	Hennessy P ₂	0.74	2.52		Hennessy P ₂	0.57	2.15
	Optical P ₂	0.76	2.49		Optical P ₂	0.60	2.18
	Photo B LM%	0.84	2.00		Photo A LM%	0.67	1.77
Leg LM %	LM% (carcass)	0.89	1.33	Belly LM%	LM% (carcass)	0.80	2.51
	Loin C LM%	0.69	2.29		Loin C LM%	0.63	3.51
	Ulster Probe P ₂	0.63	2.25		Ulster Probe P ₂	0.63	2.93
	Hennessy P ₂	0.61	2.23		Hennessy P ₂	0.62	3.03
	Optical P ₂	0.63	2.29		Optical P ₂	0.64	3.01
	Photo A LM%	0.65	2.17		Photo A LM%	0.72	2.95

Conclusion

The most accurate prediction of LM% of primal cuts is based on the lean meat percentage of the whole carcass. Magowan *et al.* (2009) showed that lean meat percentage of the whole carcass can be accurately predicted using back fat depth at P₂. Furthermore, equations using back fat depth at P₂ as measured using the Ulster, Hennessy or Optical grading probes, are accurate to predict (from RMSEP) the lean meat percentage of the loin, shoulder and leg, but not the belly.

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Computer-assisted image analysis for the comparison of sampling methods for assessing wool properties in Arkharmerino × Ghezel crossbreed sheep

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Introduction Usually investigations on wool properties are carried out using classical instruments like a Projection Microscope at wool laboratories in Iran. It is readily acknowledged that the Projection Microscope is of limited usefulness. Firstly, it is a very slow and labour intensive technique. Secondly, it is very imprecise when a single operator conducts measurements within one laboratory. Image analysis presents a quick, reliable, unbiased technique for determining wool properties (Rose and Debra, 2001). The application of computer-assisted image analysis to the measurement of wool properties potentially enables a significant reduction in the counting time for each sample. Recently in Iran, coarse-mixed wools have been valued and used in similar areas as finer wools. Coarse-mixed wools can be blended with artificial fibres to produce high valued clothes. They are also used widely in carpet production. This is why, in Iran, carpet wool breeds of increasing fleece weight is a main selection target, with improvement of fineness and uniformity as the next most important. Typically small samples from the whole fleece are taken using a scissors. The samples can be taken from six regions, i.e. neck, back, hip, shoulder, flank and rump, however usually they are taken from three regions, namely the shoulder, last rib and rump, in breeds with coarse-mixed wools. These sampling methods cause loss of time and require more labour. In this research, two methods of sampling the Arkharmerino × Ghezel fleece were compared. Sampling from only over the right mid-side, as occurs in fine wool breeds was compared with taking samples from three regions (shoulder, last rib and rump).

Materials and methods Wool samples were collected from 55 Arkharmerino × Ghezel crossbreed ewes with different ages that were managed at Khalat Poshan Research Station in Tabriz University. In the station shearing was done once a year, usually at the end of June. Before shearing, wool samples weighing 50g were taken from three regions of the animal, namely shoulder, last rib and rump, and from only the right mid-side in order to compare the methods. Measurements for the various wool characteristics were carried out at the Animal Fibre Technology Ltd, Tabriz University. Staple length, fibre diameter, fibre diameter variability, kemp(%) and medulation(%) were determined according ASTM D2130-90 for fibre diameter and ASTM D2968-95 for kemp and med fibres. The physical components of the instrument for doing measurements consisted of a web maker, an image acquisition system and a computer for image processing. Proprietary software was used to analyse fibre images stored in computer memory. The paired t-test was used for comparison between two methods of sampling. Statistical analyses were made using SAS software package.

Results Wool properties of Arkharmerino × Ghezel ewes for three regions (shoulder, last rib and rump) and right mid-side were given in Table 1. There was no effect of sampling method on any of the wool characteristics measured in Table 1 ($P>0.05$). The averages of staple length were longer than those reported by Farahvash *et al.* (2007) but were similar to that reported by Mokhber (2006) for Arkharmerino and Ghezel breeds and their crosses. Fibre diameter values were similar to that reported by Mokhber (2006) but lower than those reported by Farahvash *et al.* (2007) for Ghezel sheep.

Table 1 Wool properties of Ar×Gh ewes sampled at either three regions or the right mid-side.

Fleece Properties	Sampling Method			
	Three regions		Right mid-side	
	Mean ±Se	CV (%)	Mean ±Se	CV (%)
Staple length (cm)	11.40±0.42	25.56	11.10±0.38	19.58
Fibre diameter (µm)	24.30±0.55	10.50	24.93±0.51	10.80
Fibre diameter variability	32.17±0.78	22.77	32.47±0.66	22.03
Kemp (%)	1.55±0.32	28.2	1.59±0.44	28.5
Medulation (%)	2.67±0.44	26.21	2.70±0.41	26.25

Conclusions The use of Image Analysis for measuring wool properties is more efficient than Projection Microscope measurement, with a sample taking on average 30 min to measure using IA in comparison to 90 min with a Projection Microscope measurement. The results of this study would support the use of computer-assisted image analysis for the fast and accurate measurement of wool properties, where the IA system has been thoroughly verified to ensure accurate and repeatable results. All the differences between the mean values of the samples from two methods were statistically non significant. The right mid-side method is suggested as the preferred sampling method.

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Influence of abattoir, carcass suspension method, ageing time and anatomical position of the measurement on visual and near infrared spectrum characteristics of the *longissimus dorsi* of lambs

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Introduction Visual and near infrared reflectance (VisNIR) has been identified as a possible on-line method that could discriminate some eating quality attributes of lamb (Andres *et al.* 2007). However, the accuracy and repeatability of this method for predicting eating quality depends on the development of an optimal sampling protocol (Shackelford *et al.*, 2004) and of an optimal formula for the prediction model. The aims of this experiment were to identify if factors such as abattoir, carcass suspension method, ageing time and anatomical position along the *Longissimus dorsi* muscle can affect the characteristic of the VisNIR spectra. The information obtained from this study will allow the development an optimal protocol and prediction model.

Materials and methods The experiment used a total of 66 lamb carcasses collected from two abattoirs (X and Y). All lamb carcasses from abattoir X (n=46), were hung by the achilles, but in abattoir Y (n=20), ten carcasses were hung by achilles and ten were hip suspended. Samples of *longissimus dorsi* (LD) between vertebrae 21 and 26 were removed 2 days post slaughter and vacuum packed. Samples from abattoir X were opened in the laboratory 3 days post slaughter, allowed to bloom for 30 minutes and then reflectance was measured on the surface of the LD at the vertebrae 21 (V21) and 26 (V26) from 350 to 2500 nm using a contact probe with 10mm measurement spot (Analytical Spectral Device, ASD) coupled to an Agri Spec spectrometer (ASD). After the reflectance was measured, the LD was vacuum packed and stored at 2°C until seven days post slaughter when the reflectance measurements were repeated as for day 3. A similar procedure was used for the samples for abattoir Y except that VisNIR measurements were taken at 7 and 10 days post slaughter. The spectrum obtained were analysed by Principal Component Analysis (PCA) using Genstat Software. The analysis was done in four sets: S1; X abattoir, 2 ageing time (3 and 7 days), both positions (V21 and V26). S2; Y abattoir, two ageing times (7 and 10 days), both positions (V21 and V26) and both suspension methods (Achilles and hip). S3; both abattoirs, 3 ageing times (3, 7 and 10 days), position V21, achilles suspension. S4; both abattoirs, 3 ageing times (3, 7 and 10 days), position V26, achilles suspension.

Results For all 4 data sets around 80% of the variation was explained by the first 2 components (PC1 & PC2), with around 90% of the variance being explained by the addition of a third component (PC3). Set S1 (abattoir X), showed a clear separation between ageing times 3 days (A & B) and 7 days (C & D) along PC3 (see figure 1). However, no separation in the distribution of the samples was observed for muscle measurement position, either on plot PC1/PC2 or on plot PC1/PC3. In set S2 (abattoir Y), there was no clear separation between aging times 7 and 10 days; however, there was indication that PC1 may show separation in relation to hanging method. When the data was analysed for each position but with both abattoirs combined (set S3 position V21 and set S4 position V26) there was no clear separation between treatments in PC1/PC2; however, ageing time 3 and 7 days (abattoir X) and 3 and 10 between both abattoirs, gave a clear separation on cluster on PC1/PC3. No separation was identified between ageing time 7 and 10 for the abattoir Y in either set S3 or S4.

Figure 1 PC1 and PC3 of VisNIR measured for samples of X abattoir (S1). A: V21 ageing 3 days; B: V26 ageing 3 days; C: V21 ageing 7 days; D: V26 ageing 7 days

Conclusion On the basis of this preliminary study, it appears that the VisNIR spectrum is affected by ageing times, especially between 3 and longer ageing times. This suggests that different prediction models would be required depending the time post slaughter when VisNIR measurements are made. However, it also appears that VisNIR prediction models may be applicable across different abattoirs and muscle positions. Further work is required to evaluate the effect of the hanging method on VisNIR spectra.

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Evaluation of the direct effects of MyoMAXTM on carcass traits in crossbred lambs

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Introduction Quantitative trait loci (QTL) located on chromosome 2 in the region of the myostatin gene or polymorphisms in the myostatin gene have shown major effects on sheep carcass muscling (e.g. Clop *et al.*, 2006). MyoMAXTM (MM-QTL) is one such QTL/allele identified in Texel sheep, which increases carcass muscling and reduces carcass fatness (e.g. Laville *et al.*, 2004; Johnson *et al.*, 2005). The UK sheep industry may benefit from using MM-QTL to increase meat yield in lamb carcasses once its effects are verified in a genetic background typical of the stratified UK system.

Materials and methods Multiparous Mule (Bluefaced Leicester × Scottish Blackface) ewes were inseminated with semen from 2 Texel rams that were heterozygous for MM-QTL, to generate 150 lambs that were either carriers (n=70) of a single copy of MM-QTL or non-carriers (n=80). Lambs were weighed and scanned using X-ray computed tomography (CT) at ~120 days old to measure fat, muscle and bone areas in three cross-sectional CT scans; *Ischium* through the hind leg, *LV5* through the loin and *TV8* through the shoulder/chest. Tissue areas and live weight at scanning were used to predict total carcass fat, muscle and bone weights, which were then used to calculate tissue weights and proportions. Lambs were weighed and ultrasound scanned at ~150 days old, to measure muscle and fat depths over the 3rd lumbar vertebra, and then slaughtered. Carcasses were weighed and classified for fatness and conformation based on the MLC classification scheme (for analysis, fat class was transformed to estimate subcutaneous fat%; Kempster *et al.*, 1986). Carcasses were then scanned using a video image analysis (VIA) system and standard and refined VIA prediction equations (Rius-Vilarrasa *et al.*, 2009) were applied by the VIA providers (e+v technology GmbH, Germany) to obtain predicted weights of primal cuts and trimmed primal cuts (shoulder, breast, leg, chump, loin) and predicted weights of carcass saleable meat yield and trimmed saleable meat yield. Data were analysed using GLM in SAS (SAS Institute Inc., Cary, NC, USA) to determine the effects of MM-QTL. Fixed effects fitted were MM-QTL carrier status and sex. Sire was fitted as a random effect and either live weight or carcass weight (for in-vivo and post-slaughter measurements, respectively), as a covariate.

Results Muscle areas (MA) in the three CT scans were significantly higher in MM-QTL carrier lambs (2.7 to 4.3%; Table 1). Areas of fat (although 4-9% lower) and bone were not significantly affected by MM-QTL carrier status. Although not significant, there was a trend for MM-QTL carrier lambs to have a higher CT predicted muscle weight (1.7%) and muscle proportion (1.2%) and lower fat weight (-4.5%) and fat proportions (-4.7%) than non-carriers. The tendency for carriers to be less fat was supported by carcasses from MM-QTL carrier lambs being classified as significantly less fat by the MLC classification scheme. MLC carcass conformation score was not affected. Using either standard or refined algorithms, VIA did not detect any significant effect of MM-QTL on weights of saleable meat yields or primal cuts in the carcass.

Table 1 Least square means for MM-QTL carrier and non-carrier lamb groups for some of the measured traits.

	CT scanning					MLC
	Ischium MA(cm ²)	LV5 MA(cm ²)	TV8 MA(cm ²)	Muscle wt (kg)	Fat wt (kg)	Fat Class
Non-carrier	242.18	79.63	109.91	10.97	3.00	10.62
Carrier	248.70	83.03	113.04	11.15	2.87	9.92
s.e.d	21.0004	10.897	15.270	0.0177	0.0158	0.016
P-value	0.0004	0.0004	0.018	0.204	0.289	0.015
%difference	2.7	4.3	2.8	1.7	-4.5	-6.6

Conclusions MM-QTL effects were visible in a few key carcass traits in the crossbred lambs studied, with lambs carrying a single copy of MM-QTL tending to be leaner than non-carriers. However, differences were relatively small in size and not always statistically significant. VIA could not detect any differences between MM-QTL carrier and non-carriers with either standard or refined prediction equations. Observed effects of the QTL on fat and muscle traits in this study, under UK conditions, were similar in direction and magnitude to those reported previously for leg composition traits in crossbred lambs in New Zealand (Johnson *et al.*, 2005). It is of note that mutations in the myostatin gene are usually recessive, so that larger effects could be expected in homozygous carriers.

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Isomerisation of trans-10 cis-12 conjugated linoleic acid during acidic methylation

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Introduction Conjugated linoleic acid (CLA) has been associated with numerous health benefits in animal models including modulation of immune response and fat deposition (Lock & Bauman, 2004). The most predominant CLA in ruminant products (meat and milk) is cis-9 trans-11 with traces of other isomers such as trans-10 cis-12 which can be increased according to dietary regimes such as increasing the proportion of concentrate in the diet (Sackmann *et al.* 2003). More recent findings into the health benefits of CLA in humans have found the trans-10 cis-12 isomer to be more potent than cis-9 trans-11 (Tholstrup *et al.* 2008). Whilst it has been reported that acidic methylation during analysis of milk fat results in isomerisation of cis-9 trans-11 (Lee & Tweed, 2008) little information is available on the effect of different methylation regimes on the increasingly important trans-10 cis-12 isomer. This study investigated the isomerisation of esterified and un-esterified trans-10 cis-12 CLA during three methylation regimes.

Materials and methods Two known trans-10 cis-12 CLA certified standards (10-1826-90-13, 90% CLA 10tr,12c Free Fatty Acid; and 33-1826-90-13 90% CLA 10tr, 12c Triglyceride, Larodan Fine Chemicals, Malmo, Sweden) were methylated in triplicate using three methylation regimes: i) acidic methylation (AM; Sukhija & Palmquist, 1988) – 10 mg of CLA standard in 2 ml of toluene containing 0.4 mg/ml C_{23:0} as an internal standard. The methylation mixture and conditions consisted of 3 ml of a mixture of freshly made acetyl chloride to ten parts methanol at 70°C for 2 h; ii) bimethylation (BiM; Kramer & Zhou, 2001) - 10 mg of CLA standard resuspended in 1 ml of n-heptane containing 1 mg C_{23:0}. The methylation mixture and conditions consisted of 4 ml of NaOH in methanol (0.5 mol/l, 15min) followed by 4 ml of HCl in methanol (1.4 mol/l, 1h) both incubated at 50°C; iii) base methylation (BM; Lee & Tweed, 2008) - 10 mg of CLA standard resuspended in 1 ml of n-heptane containing 1 mg C_{23:0}. The methylation mixture and conditions consisted 4 ml of NaOH in methanol (0.5 mol/l, 15min) at 50°C. Fatty acid methyl esters (FAME) were analysed by gas liquid chromatography on a CP-Select chemically bonded for FAME column (100 m × 0.25 mm ID, Varian Inc, California, USA.) with split injection (30:1) and He as the carrier gas. Identification of CLA isomers was performed using a commercially available CLA methyl ester standard (O-5632, Sigma-Aldrich, St Louis, Missouri, USA). Data was analysed using a general ANOVA with methylation regime as the treatment effect (Payne *et al.* 2002).

Results Figures 1 and 2 show the fatty acid profiles of the un-esterified and esterified CLA standards, respectively. The BM is unable to methylate un-esterified fatty acids and is not shown. AM resulted in significant isomerisation of trans-10 cis-12 CLA in both standards with consequent increases in cis-9 trans-11 CLA, trans-trans CLA and unidentified fatty acids. Both BM and BiM of the esterified standard and BiM of the un-esterified standard resulted in full recovery of the trans-10 cis-12 isomer as determined by the manufacturers certified contents certificate.

Figure 1 Fatty acid profile of the un-esterified standard

Figure 2 Fatty acid profile of the esterified standard

Conclusions Trans-10 cis-12 CLA when esterified or un-esterified is isomerised during acidic methylation into trans-trans CLA and other unidentified fatty acids. Methylation of samples in CLA studies should be carried out using base or bimethylation procedures.

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

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Introduction It is common practice to give cereal fed bulls a 160g/kg crude protein (CP) diet to 250kg live weight, dropping to 140g/kg CP from 250kg to slaughter. The majority of intensively fed beef cattle are fed home mix rations based on rolled barley with a protein concentrate or 'protein rich' straights such as rapeseed meal and soya bean meal. In this latter situation higher protein rations will have increased ration costs. The objective of this experiment was therefore to determine the effect of feeding barley based rations containing 120, 140 or 160g/kg dietary CP on the performance of cereal fed 280kg Holstein bulls through to slaughter.

Materials and methods Forty eight Holstein bulls weighing 280kg were reared through to slaughter on a cereal beef system and fed *ad libitum* diets containing either 120, 140 or 160g/kg dietary CP. The 120g/kg CP ration contained the following ingredients (g/kg): rolled barley 755, soyabean meal 37.5, rapeseed meal 37.5, molassed sugar beet pulp 100, molasses 50, and mineral 20. The 140 and 160 g/kg CP diets were formulated with 140 and 200g/kg soyabean meal and rapeseed meal (50:50) replacing an equal quantity of barley. The cattle were housed in straw-bedded pens and were selected for slaughter at EUROP fat class 3. The data was analysed using ANOVA.

Results Overall the bulls recorded performance that either achieved or exceeded recognised targets for cereal beef production. There were no significant differences in animal performance between the treatments.

Table 1 Animal Performance

Crude protein (g/kg)	120	140	160	s.e.d	Sig
Slaughter weight (kg)	557	549	542	11.2	NS
Days to slaughter	205.6	205.0	206.4	11.09	NS
DLWG (kg)	1.34	1.32	1.33	0.054	NS
Carcass wt (kg)	284.1	280.1	277.6	5.73	NS
Kill out (g/kg)	510	510	513	4.6	NS
Carcass daily gain (kg)	0.741	0.731	0.737	5.73	NS
Conformation class ¹	2.0	2.1	2.0	0.16	NS
Fat class ¹	3.17	3.08	3.17	0.228	NS
Carcass price (£/kg)	1.76	1.77	1.76	2.92	NS
Carcass value (£)	504	497	490	13.8	NS

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

Table 2 Feed use, Feed Conversion Ratio (FCR) and Feed cost per kg gain

Crude protein (g/kg)	120	140	160
Daily feed intake (kg)	9.22	8.90	8.89
Total feed intake (kg)	1,895	1,825	1,834
FCR (kg feed: kg LWG)	6.94	6.80	6.75
FCR (kg feed: kg carcass gain)	12.57	12.31	12.17
Feed cost (p/kg LWG)	74.5	74.7	75.8
Feed cost (p/kg carcass gain)	134.5	135.2	136.7

The bulls fed the 120 g/kg CP ration recorded the highest daily and total feed intake and the worst FCR. The FCR's (kg feed: kg LWG) appear to be relatively high compared to the target of 5:1 for cereal beef production. It must be noted that the trial did not include the period of growth from 120kg to 280kg. During this rearing phase Holstein bulls at Harper Adams typically record a DLWG of 1.75kg with an FCR of 3.2.

Conclusions There were no significant differences in animal performance between the treatments. The highest slaughter and carcass weights were achieved by the bulls fed the 120g/kg CP ration however this was not statistically significant. It has been suggested that feeding a higher protein ration could increase slaughter weight or help produce leaner carcasses but this was not evident from this trial. The excess protein from the 140 and 160 g/kg CP diets would have to be converted to urea by the rumen micro-organisms, absorbed across the rumen wall and excreted in the urine. This will demand energy which would otherwise be used for growth. The bulls reared on the 120 g/kg CP recorded the highest carcass value and lowest feed cost per kg LWG. From the experiment it can be concluded that increasing the protein content of the ration above 120g/kg CP for 280kg bulls does not improve physical or financial performance.

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Animal performance characteristics in suckler-bred steers and heifers finished at 16-20 months of age on a grass silage and barley-based concentrate ration offered as a complete diet

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Introduction Due to an increasingly discerning consumer demand, the eating quality of beef has become a major focus of research effort for the beef supply chain and some pricing schemes currently reward Aberdeen Angus sired animals over animals sired by other breeds. However, the animal performance characteristics of beef cattle at a farm level must also be examined in studies on beef eating quality since they will remain an integral part of primary producer returns for the foreseeable future. As part of a wider beef eating quality project, the objective of the current study was to assess the on-farm and carcass animal performance characteristics in suckler-bred steers and heifers during the finishing phase.

Materials and methods A total of 100 steers and heifers were used in a continuous factorial design experiment where two breed types (B) and two sexes (S) of suckler-bred finishing cattle were compared. The two breed types were either Aberdeen Angus x Limousin (AAx) or Limousin x Aberdeen Angus (LIMx) derived from the reciprocal crossing programme within the main SAC beef herd. There were 27 AAx steers, 31 LIMx steers, 21 AAx heifers and 21 LIMx heifers allocated to 3 pens per sex on the basis of initial LW and days of age. All animals were fed the same finishing diet consisting of 1st cut grass silage and a barley based concentrate (50:50 DM basis) which was offered as a completely mixed ration on a daily basis (DM: 383 g/kg, ME: 12.2 MJ/kg DM, CP: 140 g/kg DM). All animals remained on the diet for a minimum of eight weeks after which they were selected for slaughter according to standard commercial practice (target grades R4L or better). Animal liveweight (LW) was recorded weekly throughout and daily liveweight gain (DLWG) figures determined by linear regression. Carcass weight was estimated at the start of the experiment (Keady & Kilpatrick, 2005) and cold carcass weight gains (CCWG) determined as the difference between initial CCW and CCW at slaughter. Fat and conformation gradings were also recorded at slaughter and converted to a 1-15 point score for statistical analysis. Killing out proportion (KO prop) was also calculated from LW and CCW at slaughter. Data was analysed for the fixed B, S and BxS effects and the random within S pen effect using the REML facility in Genstat 8.

Results Adjusted means for the animal performance characteristics (age at slaughter, LW at the start of the experiment and at slaughter, DLWG, CCW at the start of the experiment and at slaughter, daily CCWG, KO prop, fat and conformation numerical scores) are given in Table 1. Breed type and animal sex along with their inter-action were all significant effects in all parameters except age at slaughter where only the BxS inter-action was significant. Only the breed type and its inter-action with sex were a significant effect for conformation score. In general, LIMx animals grew faster, had a higher KO proportion and produced heavier carcasses with a higher conformation and lower fat scores compared to AAx animals. Similarly, steers generally grew faster, had a higher KO proportion and produced heavier carcasses with a lower fat score compared to heifers. AAx animals achieved a commercial price premium averaging 4.6 pence/kg CCW but due to the carcass characteristics listed above, overall carcass value was £28.90 per head less compared with the LIMx animals.

Table 1. Animal performance characteristics during the finishing phase and at slaughter in suckler-bred steers and heifers

Performance characteristic	AAx		LIMx		Breed (B)		Sex (S)		s.e.d's			Sig of effects		
	St's	Hf's	St's	Hf's	AAx	LIMx	St's	Hf's	B	S	BxS	B	S	BxS
Age @ slaughter (days)	544 ^{ab}	549 ^{ab}	536 ^a	551 ^b	546	544	540	550	5.5	5.1	6.7			*
LW @ start (kg)	481 ^b	462 ^c	506 ^a	474 ^{bc}	472	490	494	468	4.1	6.9	7.5	***	***	*
LW @ slaughter (kg)	601 ^b	563 ^c	631 ^a	588 ^b	582	609	616	575	4.0	7.9	8.3	***	***	**
DLWG (kg/d)	1.56 ^a	1.25 ^b	1.57 ^a	1.46 ^a	1.40	1.52	1.57	1.36	0.040	0.065	0.072	**	**	**
CCW @ start (kg)	258 ^b	246 ^c	272 ^a	253 ^{bc}	252	263	265	250	2.4	4.1	4.4	***	***	*
CCW @ slaughter (kg)	331 ^b	303 ^c	357 ^a	323 ^b	317	340	344	313	2.6	5.5	5.7	***	***	***
CCWG (kg/d)	0.93 ^{ab}	0.65 ^c	0.99 ^a	0.86 ^b	0.79	0.93	0.96	0.76	0.010	0.036	0.040	***	***	**
KO prop (g/kg CCW)	554 ^{ab}	537 ^c	565 ^a	551 ^b	546	558	560	544	3.8	3.8	5.3	**	***	*
Fat score (1-15)	9.10 ^b	10.76 ^a	8.46 ^b	8.90 ^b	9.93	8.68	8.78	9.83	0.244	0.341	0.390	***	***	***
Conf score (1-15)	8.00 ^a	7.98 ^a	8.78 ^{ab}	8.89 ^b	7.99	8.84	8.39	8.44	0.289	0.359	0.425	**		*

Values within the BxS inter-action not sharing superscripts differ significantly (P<0.05).

Conclusions These results show that LIMx animals had higher carcass gains and produced heavier, better conformed carcasses compared to AAx animals. Whilst AAx animals did achieve higher prices per kg carcass weight, their basic animal performance characteristics resulted in lower financial values overall. These fundamental commercial performance characteristics should be taken into account when considering the economic sustainability of future beef production systems where pricing structures may be further developed to reward beef eating quality parameters.

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Effect of weaning dairy-bred bull calves either gradually or abruptly

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Introduction There are four main parameters to determine weaning time with artificially reared dairy-bred calves: age, liveweight, concentrate intake and milk price. Most commercial calf rearers use concentrate intake as the main method weaning calves when eating 1kg of concentrates per day. Calves can be weaned either abruptly or gradually where the quantity and strength of milk replacer is reduced over a period of time. The objective of this experiment was to evaluate the effect of gradual or abrupt weaning strategies for calves weaned at 42 days of age.

Materials and methods Thirty six Holstein and Continental cross Holstein bull calves were assigned in a randomised block designed experiment to weaning at 42 days of age either gradually or abruptly. The calves started the trial at 1 day of age and were individually penned on straw. From days 1 to 3 they were fed colostrum. The calves were fed a whey based milk replacer (DM 954g/kg, 241g crude protein/kg, 201g/kg Ether Extract [Wynngold Bloom Milk Replacer, Wynnstay Group plc]) mixed at 125g per litre of water at 40°C twice per day in buckets. From 4 to 7 days they were fed 4 litres per day and from day 8 milk was fed at 5 litres per day. The abruptly weaned calves were weaned at 42 days of age. The gradually weaned calves were fed a reduced rate of milk replacer from 1 week prior to weaning. The milk replacer was gradually reduced from 5 litres to 1 litre per day (1 litre reduction every 2 days) by weaning at 42 days old. From day 4 the calves on both treatments received *ad libitum* concentrates (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 gradually weaned calves recorded significantly higher ($p < 0.05$) daily live weight gain (DLWG) from weaning to 11 weeks and had an improved coat bloom score ($p < 0.01$) at 11 weeks of age.

Table 1 Effect of weaning strategy on liveweight (kg) and coat bloom score

	Abrupt	Gradual	s.e.d	Sig
Start weight	47.2	47.2	3.04	NS
Weaning weight	62.0	62.7	3.53	NS
11 week weight	96.8	105.4	5.98	NS
Coat bloom score at 11 weeks old*	3.31	3.59	0.103	**

* Coat bloom score scale of 1 = dull, 3 = normal, 5 = shiny

Table 2 Effect of weaning strategy on DLWG (g)

	Abrupt	Gradual	s.e.d	Sig
Start - weaning	353	369	79.1	NS
Weaning - 11 weeks	993	1220	96.2	*
Start - 11 weeks	644	755	81.8	NS

Table 3 Feed intakes (kg/head)

	Abrupt	Gradual	s.e.d	Sig
Conc intake (start - weaning)	12.7	16.7	3.18	NS
Conc intake (start - 11 weeks)	93.3	107.3		
Milk replacer	23.5	21.0		
FCR (kg feed: kg gain)	2.35	2.20		

It was noted that abruptly weaned calves (mainly those that were eating less than 1kg of concentrates prior to weaning) were more vocal than gradually weaned calves, spent more time standing at the front of the pen and taking interest in humans for several days and up to a week after weaning. There were however no differences in the health or number of medication treatments between the groups.

Conclusions The gradually weaned calves were on target to achieve the MLC (1999) growth target for rearing bull calves to 12 weeks old of 115kg. The abruptly weaned calves would not achieve this target. The gradually weaned calves recorded significantly higher ($p < 0.05$) DLWG's from weaning to 11 weeks old and gained an extra 8.6kg live weight to 11 weeks of age and had an improved coat bloom score ($p < 0.01$). The gradually weaned calves consumed 11.5kg more feed and recorded an improved FCR. Total feed costs to 11 weeks old were similar however due to the improved performance of the gradually weaned calves they recorded reduced feed costs per kg gain of 16p (£0.95 v £1.11). If the additional 8.6kg of live weight gain is valued at £1.40/kg this is worth £12.04 per calf. It is concluded from this experiment that calves should be gradually weaned.

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Evaluation of barley distillers' grains ensiled with or without molassed sugar beet pulp on feed intake and production of Holstein dairy cows

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Introduction Agro-industrial by-products are characteristically high in fiber and low in starch with some disparity in protein content based on source and processing method. Barley Distillers' Grains (BDG) consists largely of structural and protein remaining when extracted residues of grains used in the brewing process. Fresh BDG contain about 70%-80% moisture and can store as silage. However, there is limited research information available on improving BDG silage with readily fermentable carbohydrate. McKendrick *et al.* (2003) ensiled BDG with molassed sugar beet pulp (MSBP) very well. The aim of this experiment was to evaluate the potential of ensiled wet BDG with and without MSBP as alternative to maize silage on DM intake, milk yield and composition of dairy cattle.

Materials and methods Eighteen Holstein cows (7 primiparous and 11 multiparous with average of 86 d in milk) were assigned in to 3 treatments of six cows. Dairy cattle were selected according to body weight and milk yield on the week prior to the start of experiment. Treatments comprise of ensiled BDG with different level of MSBP and provided as followed: A) 40% MSBP+60% BDG (DM basis). B) 20% MSBP+80% BDG and C) 0% MSBP+100%BDG. Experimental diets contained, 40% forage (60:40 whole maize silage: Lucerne hay) and 60% concentrate. The treatments of ensiled BDG (A, B and C) were substituted with 30% (DM basis) maize silage. The experiment consisted of four periods each of 2 weeks with collection of data in the last week of each period. Throughout the experiment, cows were housed in tie stalls and fed three times daily after milking (6:00 h, 14:00 h and 22:00 h). The data were examined by analysis of variance based on completely randomized design (Repeated Measures) using SAS (9.1).

Results Effect of BDG ensiled with and without MSBP on DM intake, daily milk yield and composition represented in Table 1. Murdock *et al.* (1981) fed rations in which wet brewers' grains constituted 0-30 % of the ration on a dry matter (DM) basis without any significant decline in milk production. No significant differences were seen in DMI, milk yield and composition between diets. Results show that treatment C had a numerically lower DMI intake than the other treatments. These results tended to more efficient with increasing the level of MSBP in silage; however, the difference between diets is not statistically significant. Milk fat, protein and lactose yields were higher when diet B was offered. There were high significant differences between periods ($P < 0.01$).

Table 1 Effect of Barley Distillers' Grains ensiled with or without molassed sugar beet pulp on dairy cow performance

Items	Treatments			Treatment effect		Period effect
	A	B	C	s.e.m.	P	P
DMI (kg d ⁻¹)	20.90	20.00	19.20	0.88	0.41	***
Milk yield (kg d ⁻¹)	33.68	33.21	32.56	2.76	0.95	**
Milk composition (g d ⁻¹)						
fat	762.60	880.91	770.30	61.50	0.34	0.22
Protein	998.57	1061.82	1005.82	81.35	0.83	***
Lactose	1564.69	1603.33	1554.89	119.94	0.95	***

a, b, c Means in row with different superscript letter are different.

P < 0.01, *P < 0.001.

Conclusions Based on effective NDF, the NRC (1989) recommends that at least 75% of total dietary NDF should come from forage. Firkins (1997) summarized the literature and determined that non forage NDF concentrations below approximately 15% depressed DMI by lactating cows. Under the circumstance of this experiment, the replacing of 30% maize silage with BDG ensiled with MSBP in the current experiment did not reduce DM intake and milk yield compared with BDG ensiled without MSBP. This may be due to the presence of readily fermentable non structural carbohydrate in diet and as well it's potential as an absorbent material in the ensiled material. Several possible explanations for this observation may be proposed. First, addition of MSBP increased the DM content of the mixture substantially which is known to increase intake. Secondly, the incorporation of MSBP with BDG improved fermentation in the silo. Further work is clearly required to establish the rumen fermentation kinetic when supplying different non structural carbohydrate in silage and diets.

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Dry matter intakes, milk yield and milk composition of dairy cows offered concentrate diets containing either yellow lupins or soya bean meal

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Introduction Lupins (*Lupinus*; *Leguminosae*) as a high protein, high energy, nitrogen-fixing grain legume, have the potential to be used as a home-grown feedstuff to replace soya (*Glycine max*) in livestock feeds in the UK. Lupins are not typically grown in the UK but their high feed value and the low alkaloid concentrations in new varieties have prompted a renewed interest in their use (Wilkins and Jones, 2000). Traditionally, white lupin (*Lupinus albus*) is the predominant species fed as a soya replacement to dairy cows in Europe and the USA but recent research has shown advantages of growing yellow lupins (*Lupinus luteus*) in the UK compared with white lupins. This study reports on the effects of feeding concentrate diets containing yellow lupin compared with soya bean meal on intakes, milk productivity and milk composition in dairy cows.

Materials and methods Twenty-four mature Holstein-Friesian lactating dairy cows were divided into two groups and offered concentrate feeds containing 32 % of either yellow lupin (cv. Wodjil) or soya bean meal. The concentrate diets (pelleted) had a target crude protein (CP) concentration of 264 g kg⁻¹ DM and a metabolisable energy (ME) value of 13.4 MJ kg⁻¹ DM. The experiment was divided into 3 phases: a covariate period (weeks 2 and 3 of lactation), an adaptation period (week 4 of lactation) and a measurement period (weeks 5 to 12 of lactation). Cows were housed as one group and fed using Hoko roughage intake control feeders. During the covariate period, *ad libitum* (10 % above the unrestricted daily consumption) ryegrass silage and 6 kg d⁻¹ of standard dairy concentrate was offered to all cows and feed intakes and milk yields were recorded. Cows were then allocated to treatment (according to calving date, parity, previous milk yield, covariate milk yield, live weight and body condition), adapted to their respective concentrate diet over 7-d and the amount of concentrate offered was increased to 9 kg d⁻¹. During the measurement period, cows received ryegrass silage *ad libitum* and concentrates of each dietary treatment (9 kg d⁻¹ animal⁻¹) were fed 3 times a day. Fresh silage and concentrates as offered were DM sampled, with sub-samples of the feed offered being bulked, freeze dried and ground for chemical analysis for each week of the measurement period. Cows were milked at approximately the same times each day and their milk yields recorded. Milk samples from two consecutive milkings were collected from all cows every week to determine milk composition. Data were analysed as repeated measures on Genstat® by REML, assuming an auto-regressive correlation structure.

Results The DM content of the concentrate diets were 846 and 851 g kg⁻¹ freshweight for the yellow lupin and soya bean meal diets, respectively. The nitrogen (N) concentration of the yellow lupin diet was 44.1 g kg⁻¹ DM and the N concentration of the soya bean meal diet was 41.1 g kg⁻¹ DM. Both N values were slightly lower than the target CP concentration in the formulations but were within an acceptable range. The ME concentration of both concentrate diets was 12.8 MJ kg⁻¹ DM. Dairy cows offered the soya bean meal concentrates had a higher DM intake, and therefore, ME intake than cows offered the lupin dietary treatment but there was no difference in the N intake by cows on the two treatment diets. As animals on both dietary treatments consumed 9 kg of concentrates each day, the intake data showed that dairy cows offered concentrates containing soya bean meal consumed more ryegrass silage than those offered concentrates containing yellow lupins. There were no significant differences in the milk yield or milk composition of dairy cows offered concentrate diets containing either yellow lupins or soya bean meal during weeks 5 to 12 of lactation (Table 1).

Table 1 Total dry matter (DM), metabolisable energy (ME) and nitrogen (N) intakes and milk yield (kg d⁻¹) and milk composition (g kg⁻¹) of dairy cows offered *ad libitum* ryegrass silage and 9 kg d⁻¹ of concentrates containing either yellow lupins or soya bean meal during weeks 5 to 12 of lactation.

	Yellow lupins	Soya bean meal	s.e. of difference	Level of significance
Milk yield	30.1	31.9	1.35	ns
Fat	39.8	37.4	1.42	ns
Protein	28.9	29.9	0.54	ns
Lactose	46.4	45.9	0.36	ns

ns, not significant.

Conclusion Overall, the findings from this study indicate that yellow lupins could be used as a home-grown alternative feed grain source to imported soya in dairy diets in the UK. Further studies are now needed to determine the long-term effects of using yellow lupins within UK dairy production.

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Effect of zinc sources on haematology, enzyme activity and blood serum zinc concentration in Holstein dairy cattle

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Introduction Zinc (Zn) has a wide spectrum of biological activities and its deficiency has been related to various dysfunctions and alterations of normal cell metabolism (El Hendy *et al.*, 2001). This element is a central component of hundreds of metalloenzymes which are concerned with the metabolism of feed constituents. Zn requirements of ruminants appear to be affected by dietary factors based on the variable animal responses that were observed after zinc supplementation. Supplemental zinc is usually added to animal diets in the form of zinc oxide or zinc sulphate. Recently, organically bound zinc supplements are using in diets of animals. Some researchers (Wedekind *et al.*, 1992) have reported higher bioavailability for organically bound zinc sources than inorganic forms. Therefore, the objective of this experiment is to comparison the effects of organically bound and inorganic zinc supplements on haematological and biochemical parameters in blood serum.

Materials and methods Eighteen multiparous Holstein cows randomly allocated to one of three groups. Zinc content of the control diet was 42 mg/kg diet DM. Treatment groups received the basal diet with no supplemental Zn (control), basal diet plus 500 mg of Zn/ kg of DM as ZnSO₄ .H₂O (ZnS) and basal diet plus 500 mg of Zn/kg of DM as Zinc methionine (ZnM). Animals were housed in individual stalls and fed the experimental diet as a TMR at 0800 and 2000h. This experiment was started in the first phase of lactation (35±3 days after parturition). Plasma and serum were taken from samples of blood on 0, 2, 4, 6, 8 and 10 weeks of experiment. Anti-coagulated blood was analysed shortly for number of white blood cell (WBC), number of red blood cell (RBC), haemoglobin (Hb), packed cell volume (PCV), mean Corpuscular Volume (MCV), mean Corpuscular Haemoglobin (MCH), mean Corpuscular Haemoglobin concentration (MCHC) and number of platelet (PLT) by an automatic haematology cell counter. Serum samples were stored at -20°C. Stored serum samples were also analysed for lactate dehydrogenase (LDH), alkaline phosphatase (ALP), super oxide dismutase (SOD) and zinc concentration. The data were analyzed using the mixed procedure of SAS (9.1) for a completely randomised design with repeated measures.

Results The results of haematological parameters and serum zinc concentration are shown in Table 1. The number of red blood cell, haemoglobin, MCHC, number of platelet and serum zinc in the ZnM and ZnS groups were significantly higher than control group ($P < 0.05$). Mentioned factors were also higher ($P < 0.05$) in dairy cattle that received ZnM than ZnS. There were no differences for WBC, PCV, MCH and PLT. There were not significant differences among different sources of Zn for ALP, but LDH and SOD in ZnM group were significantly higher than ZnS (Table 2).

Table 1 Effects of Zn sources on haematological parameters and serum zinc of three groups

Parameter	C	ZnS	ZnM	SEM	P
WBC (/μL)	9055.3	8467.8	9114.3	377.6	0.14
RBC (10 ⁶ /μL)	6.07 ^{ab}	5.88 ^b	6.38 ^a	0.22	0.03
Hb (g/dL)	8.33 ^b	8.25 ^b	8.83 ^a	0.21	0.04
PCV (%)	25.81 ^b	25.50 ^b	27.60 ^a	0.38	0.002
MCV (fL)	42.35	44.14	43.54	0.91	0.40
MCH (pg)	13.65	14.07	13.93	0.36	0.29
MCHC (g/dL)	31.67 ^b	32.56 ^a	32.76 ^a	0.30	0.05
PLT (10 ³ /μL)	382233	382762	427946	27561	0.60
Serum Zn (μg/ml)	1.33 ^c	2.84 ^a	2.43 ^b	0.12	<0.0001

Table 2 Effects of Zn sources on some enzyme activities

Parameter	C	ZnS	ZnM	SEM	P
LDH (IU/L)	704.2 ^b	733.57 ^a	740.9 ^a	11.57	0.05
ALP (IU/L)	98.87	101.19	108.68	8.15	0.68
SOD (IU/L)	118.31 ^b	140.51 ^a	144.61 ^a	1.51	<0.0001

Conclusions Supplementation with high concentration (500 mg/kg) of zinc resulted in elevated concentration of Zn in blood serum, some of haematological parameters and enzyme activity of LDH and SOD which is consisted with previous studies (Wright and Spears, 2004). Data obtained from this study indicated that organically bound zinc had higher effect on some blood parameter and enzyme activities of LDH and SOD.

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Effect of different sources of supplemental protein on performance and on ruminal pH and N-NH₃ of Holstein dairy cows

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Introduction Pistachio hull (PH) is the main pistachio by-product produced from the pistachio dehulling process. The product consists of 12.7, 5.7, 16.6, 25, and 20% ash, EE, CP, NDF, and ADF, respectively and can be used as a feedstuff for ruminant animals. It is a mount of energy, fibre and potassium (Naserian and Vahmani, 2005). Because its constitutive tannin content can bind protein and cellulose, it was hypothesized that PH tannin can decrease the digestibility of protein. There is no clear consensus amongst researchers on the effect of dried pistachio by product on milk yield, milk composition, rumen fluid pH and N-NH₃ concentration. The main objective of this experiment was to evaluate effects of different sources of supplemental protein with dried pistachio by product on milk yield, milk composition rumen fluid pH and N-NH₃ concentration of dairy cows.

Materials and methods Eight multiparous Holstein cows in early lactation (80 ± 10 days postpartum, weighing 600 ± 20 kg) were assigned into a replicated 4×4 Latin square design with 3-wk periods. The dietary treatments were as follows: T1) 18% alfalfa, 20% corn silage base on soya bean meal (19 %); T2) 18% alfalfa, 10% PH replacement of corn silage base on soya bean meal; T3) 18% alfalfa, 20% corn silage base on canola meal; T4) 18% alfalfa, 10% pistachio by product replacement of corn silage base on canola meal. Each experimental period was 21 days including 14 days adaptation period and 7 days of sample collection. Milk yield was recorded daily and milk samples were taken at each milking during the last 3 days of each period. Milk samples were subjected to analysis for CP, lactose, fat and SNF. Rumen samples were taken from each cow on the last days of each experimental period at about 3 h after the morning feeding. Ruminal fluid pH was determined immediately, and subsequently, ruminal fluid was acidified with 10 mL of HCl solution (50%, vol/vol) and stored frozen for ammonia N analysis. Data were analysed with the general linear model procedure of SAS (v. 9.01).

$$y_{ij}(k)m = \mu + SQm + ROW(SQ)im + COL(SQ)jm + t(k) + e_{ij}(k)m$$

Results The dry matter intake, milk yield and composition, ruminal pH and N-NH₃ data are presented in the Table 1. DMI, milk yield, milk composition and ruminal pH were not affected by treatments ($P > 0.05$). However, ruminal N-NH₃ was affected by treatments ($P < 0.05$). The substitution of PH with corn silage at 10 % level base on canola meal decreased N-NH₃ more than third treatment. This may have been because of PH and the tannin and phenolic components of canola. Results of the present experiment indicated that the substitution of corn silage with PH at 10 % of the diet did not effect on performance.

Table 1 Dry matter intake, milk production, and composition and ruminal pH and N-NH₃ of dairy cow

treatment	T1	T2	T3	T4	SEM
Item					
DMI (kg day ⁻¹)	20.52	21.02	20.67	20.90	0.16
Milk yield (kg day ⁻¹)	34.57	34.14	33.55	32.94	0.29
Milk composition (%)					
Protein	3.8	3.6	3.5	3.7	0.001
Lactose	4.4	4.5	4.4	4.5	0.02
Fat	3.1	3.1	3.2	3.1	0.04
SNF	11.85	11.3	11.59	11.33	0.06
Ruminal pH	6.54	6.54	6.63	6.55	0.7
Ruminal N-NH ₃ (mg/dl)	14.69 ^a	14.05 ^a	13.82 ^a	10.63 ^b	0.35

Conclusion The results indicated that substitution of corn silage with dried pistachio by product with different source of supplemental nitrogen did not affect DMI, milk yield and composition or ruminal pH. This result is in agreement with other work (Bohluli *et al.*, 2007; Naserian and Vahmani, 2005). The addition of dried pistachio by product at 10% level (dry matter basis) to the dairy cow ration will decrease the cost of milk production without any side effects on animal performance. This by product can be regarded as a source of effective fibere for the ruminant animal. However, more experiments are needed for confirmation of these finding.

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Effect of different levels of fish oil and canola oil on DMI, milk production and blood metabolites of high producing Iranian Holstein dairy cows in early lactation

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Introduction Supplemental fat sources are utilized in rations for dairy cows as a common method to increase the energy density of the diet to support energy demands for milk synthesis in early lactation (Heravi Moussavi *et al*, 2007). Although dietary fat supplementation increases the energy density of the diet, data on the relationship between the addition of dietary fat and the performance of lactating dairy cows are inconsistent because the impact of dietary fat is influenced by source of fat, method of processing, amount of fat included in the diet and stage of lactation. Canola seed which contains 40% fat is an excellent source of dietary fat high in unsaturated fatty acids and protein for dairy animals. Canola oil contains 51% oleic, 25% linoleic, and 14% linolenic acids. Fish oil (FO) also contains relatively high concentrations of two polyunsaturated fatty acids of the n-3 family: (EPA, C20:5) and (DHA, C22:6). Combination of fish oil with different plant oils has been studied previously but there are few studies which have examined the inclusion of fish oil in combination with canola oil. The aim of the present study, therefore, was to study the effects of different levels of fish oil and canola oil in early lactating Holstein cows.

Materials and methods Eight multiparous early lactation Holstein cows (42 ± 12 DIM, 40 ± 6 kg daily milk yield) were fed a total mixed ration supplemented with either 0% oil (Control), 2% canola oil (CO), 2% fish oil (FO), or 1% canola oil + 1% fish oil (CoFo), according to a double 4×4 Latin square design. Each period lasted 3 wk; experimental analyses were restricted to the last week of each period. Diets were formulated according to NRC 2001. Cows were housed in tie stalls and fed the TMR two times a day to allow 5 to 10%orts (as-fed basis), and dry matter intake (DMI) was recorded at the last week of each period. Cows were milked 3 times per day and yields were recorded at each milking. Blood samples were taken from the coccygeal vein in the last day of each period, kept on ice and centrifuged within 30 min at $3000 \times g$ for 20 min. Aliquots of serum were stored at -20°C until analysis for glucose, insulin, triglyceride, cholesterol, and serum urea nitrogen (SUN). Data were analyzed as a replicated 4×4 Latin square using the GLM procedure of SAS (2001). The model included effects of diet, period and cow. Least squares means are reported throughout and significance was declared at $P < 0.05$.

Results The results of the effects of different levels of fish oil and canola oil are present on table 1. There was no effect of diet ($P > 0.05$) on DMI, milk production, blood concentrations of glucose, urea, cholesterol, triglycerides or insulin.

Table 1 Influence of different levels of fish oil and canola oil in the diet on feed intake, milk yield and plasma metabolite concentrations.

Parameter	Treatments				SEM	p
	Control	FO	FoCo	Co		
DMI, kg/d	24.92	22.05	24.33	24.17	0.86	0.10
Milk, kg/d	34.41	32.93	34.55	34.15	0.84	0.62
Glucose,mg/dl	63.75	60.62	61.87	63.75	2.22	0.70
Cholesterol,mg/dl	192.87	211.75	207.5	194.37	8.75	0.40
Triglyceride,mg/dl	9.57	10.19	10.12	11.38	0.99	0.67
Insulin, miclu/ml	18.6	13.65	13.66	13.36	4.96	0.71
SUN,mg /dL	18.87	17.62	18.00	18.12	0.73	0.68

Conclusion Dietary inclusion of canola oil and fish oil had no effect on DMI, milk production or any blood metabolite measured. Withlock *et al.* (2002) reported similar results when fish oil and soybean oil were fed separately and in combination. Blood metabolite and insulin concentrations were also not different between diets consistent with previous studies (Khorasani and Kennelly, 1998).

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Effect of supplemented diet by sucrose and/ or starch on blood cholesterol and some hepatic enzymes in Holstein steers

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Introduction Carbohydrates are the major source of energy in diets fed to dairy cattle and usually comprised 60 to 70 percent of the total diet. They provide energy for both rumen microbes and the host animal. Sugars, starch, and other reserve carbohydrates such as fructans make up the non-structural carbohydrate (NSC) fraction and are major sources of energy for high producing dairy cattle (NRC, 2001). The fermentation of different NSC sources varies in digestion characteristics and in the profiles of organic acids produced (Hall and Herejk, 2001). Cholesterol is a structural lipid that is not required in the diet, since ample amounts are synthesized in the body. This lipid is a required component of most cells of the body. Cholesterol has received a great deal of attention in the nutritional and biochemical communities because of its strange metabolism in certain regions of the body, for example, the arteries that supply oxygen and other nutrients to the heart muscle (Brody, 1999). The aim of this study was to evaluate the effect of diets containing different type of NSC (sucrose or starch) on blood cholesterol and some hepatic enzymes namely serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) in Holstein steers.

Materials and methods Four fistulated Holstein steers (body weight 280 ± 15 kg) were assigned to a 4X4 Latin square with 21 day periods; and blood samples were collected in the last day of each periods. The basal diet contained lucerne hay, barley grain, soyabean meal and sugar beet pulp (400, 290, 190 and 50 g/kg respectively). Starch (St) or sucrose (Su) or a 1:1 mixture of starch and sucrose (St+ Su) was added to the basal diet (BD) at the rate of 70 g/kg DM. Diets were offered at 2.5 times maintenance requirements (7 kg DM/ day). Animals were fed twice daily at 0830 and 1630. Vein jugular blood samples were collected before (0.0) and 4 h after the morning feeding in tubes containing heparin and immediately were centrifuged at 3000 rpm for 15 minute. The plasma was analysed for cholesterol, SGOT, SGPT, LDH and ALP using standard procedure (Merck Vitalab Selectra, Merck, Germany). Data were analysed using the MIXED procedure of SAS (2001). Statistical model was: $Y_{ijk} = \mu + T_i + C_j + P_k + \epsilon_{ijk}$, 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 Plasma cholesterol and hepatic enzymes levels of none or NSC supplemented diets are shown in Table 1. SGPT tended ($P < 0.08$) to be lower before the morning feeding when NSC supplemented diet fed to the animal. LDH tended ($P < 0.06$) to be higher 4 h after the morning feeding when NSC was added to the diet.

Table 1 Least squares means of plasma cholesterol and hepatic enzymes of Holstein steers as influenced by diets supplemented with non-structural carbohydrate sources

Item	Treatment											
	Before morning feeding						4 h after morning feeding					
	BD	Su*	St*	Su+ St**	SEM	P	BD	Su*	St*	Su+ St**	SEM	P
Cholesterol, mg/dl	74.5	72.5	71.5	74.5	5.07	0.97	69.5	79.5	73.7	82.5	9.33	0.76
SGOT, IU/L	39.2	27.7	31.0	33.5	4.16	0.34	29.5	31.0	36.0	35.0	2.81	0.37
SGPT, IU/L	21.7	17.0	18.2	19.5	1.05	0.08	17.7	20.5	17.2	19.5	2.29	0.73
LDH, IU/L	1520	1405	1585	1672	209	0.83	1092	1615	1417	1785	145	0.06
ALP, IU/L	191.7	197.5	170.5	200.2	13.0	0.42	173.5	232.5	180.7	204.7	25.5	0.42

BD: basal diet, Su: sucrose, St: starch, Su+ St: sucrose+ starch

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

Conclusions Generally, diets used in the present study had little effect on blood cholesterol and hepatic enzyme concentrations of Holstein steers. There is considerable evidence, in both humans and experimental animals, that there is an increase in serum cholesterol, total lipids, and triglycerides when a simple sugar is substituted for a complex carbohydrate in the diet (Hirsch, 1995). It has been suggested that the elevation of SGPT and SGOT might indicate an accumulation of free fatty acids transported from blood to hepatocytes (Studer, *et al.* 1993). However, literature provided little information on the influence of dietary carbohydrates on cholesterol and hepatic enzymes in adult ruminants. Therefore, there is a need to evaluate the effect of NSC on these metabolites on ruminants.

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The effect of dietary soybean isoflavones on their concentration and total output in bovine milk

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Introduction Isoflavones belong to a large group of phytoestrogens that possess the most potent estrogenic activity. Raw soybeans contain 1.2 – 4.2 mg/g dry weight of isoflavones mainly genistein, daidzein and their conjugates (Kurzer and Xu, 1997). In ruminants, isoflavones are highly metabolized by rumen microorganisms that convert daidzein into equol and genistein into p-ethyl-phenol (Lundh, 1995). Isoflavones and their metabolites can be excreted in milk, thus, soybean products used in dairy cows feeding may represent, via milk, a source of isoflavones in the human diet. Isoflavones are particularly important due to their diverse pharmacological and antioxidant properties because they reduce the risk of several types of cancers including the breast, prostate and colon (Messina and Barnes, 1991), heart disease, menopausal symptoms (Chiechi, 1999) and bone health (Anderson and Garner, 1997).

The objective of this study was to determine the effect of dietary soybean isoflavones on their concentration and total output in bovine milk.

Materials and methods The trial was carried out on four multiparous lactating Holstein cows with mean milk yield of 27.3 kg/d (± 1.7) that were divided into 2 groups. The experiment was divided into 2 periods of 42 days (21-d preliminary period and a 21-d experimental period). Cows were fed individually twice daily *ad libitum* the diet based on maize silage, meadow hay and supplemental mixture containing either extruded full-fat soya (S) or extruded rapeseed cake (R). Feed intake was monitored daily with samples taken twice a week in the experimental periods. Cows were milked twice daily (7.00 and 17.00 h). Milk yield was recorded at each milking. In the experimental period, samples of milk were taken at each milking three times a week and analysed for basic constituents by infrared analyser (Bentley Instruments 2000, Bentley Instruments Inc., USA). Blood samples were taken into heparinised tubes from *vena jugularis* three times a week after morning milking during the experimental period. The isoflavones content in feed, milk and blood samples was analysed using HPLC-MS/MS.

Data obtained in the experiment were analysed using GLM procedure of the SAS/STAT, Version 8 according to the following model: $Y_{ijklm} = \mu + T_i + S_j + C_k(S_j) + P_l + W_m(P_l) + \varepsilon_{ijklm}$ where μ = general mean, T_i = treatment effect ($i = 2$), S_j = effect of square ($j = 2$), $C_k(S_j)$ = effect of cow within square ($k = 4$), P_l = period effect ($l = 2$), $W_m(P_l)$ = effect of week within period ($m = 3$) and ε_{ijklm} = error term. For all statistical evaluations week means were used.

Results Average content of daidzein and genistein in extruded full-fat soya was 150.9 mg/kg and 222.6 mg/kg, respectively resulting in average total isoflavones intake of 3272.2 mg/d in S. Low intake of total isoflavones in R being 57.6 mg/d was also found. Milk yield in S was higher than in R ($P < 0.001$). Concentrations of daidzein and its metabolite equol in milk were higher ($P < 0.001$) and concentration of genistein tended to be higher ($P > 0.05$) in S than in R resulting in significantly higher total output of daidzein, genistein and equol in S in comparison to R ($P < 0.001$, Table 1). The concentrations of daidzein, genistein and equol in plasma were higher in S than in R ($P < 0.001$).

Table 1 Effect of dietary soybean-derived isoflavones on their concentration and total output in bovine milk

Item	Unit	R	S	SE	P ¹
Milk yield	kg/d	21.8	23.8	0.44	0.001
Daidzein	ng/ml	12.3	16.0	1.44	0.063
Genistein	ng/ml	33.2	38.3	3.96	0.133
Equol	ng/ml	3.3	52.3	3.94	< 0.0001
Daidzein	µg/d	269.2	379.4	33.23	0.0009
Genistein	µg/d	737.9	911.6	94.36	0.0386
Equol	µg/d	72.1	1248.9	94.34	< 0.0001

Conclusion Due to the fact that soybean products are a substantial part of diets for high-yielding dairy cows, milk may represent a potential source of isoflavones in the human diet. As documented in our experiment, isoflavones, particularly equol, are present in bovine milk in low amounts in dependence on diet composition. Knowledge in the area of isoflavones occurrence in bovine milk may be useful to monitor more completely overall exposure of human to phytoestrogens.

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Effect of feeding diet containing fish oil and canola oil from transition period on milk production and components of Holstein cows in early lactation.

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Introduction There are too many physiological and nutritional factors which can influence lactation performance of early lactation Holstein dairy cows. Supplemental fat sources are utilized in rations for dairy cows as a common method to increase the energy density of the diet, especially in early lactation (Juchem *et al.*, 2007). The fat sources also have a positive effect on milk fatty acid profile. Canola seed which contains 40% fat is an excellent source of dietary fat high in unsaturated fatty acids and protein for dairy animals. Canola oil contains 51% oleic, 25% linoleic, and 14% linolenic acids. Fish oil contains relatively high concentrations fatty acids of the n-3 family. It seems that feeding a blend of different oils will have more positive productive response than individual usage of them. The aim of this study was to evaluate the effect of feeding diets containing fish oil and canola oil starting from transition period on milk production and composition of early lactating Holstein cows.

Materials and methods Eighteen multiparous Holstein dairy cows were blocked by parity, expected calving time, and previous 305-2X milk production and were randomly assigned to the dietary groups. Cows were housed in tie stalls and were allowed to exercise daily in an outside lot for 3 h. Total mixed ration were formulated according to NRC 2001 requirements, supplemented with either 0% oil (Control, n=9), or 2% oil (Supplemented, 1% canola oil and 1% fish oil, n=9). Diets were fed two times a day to allow 5 to 10%orts (as-fed basis) from four week before to seven weeks after parturition. The fat content (%DM) of diets in transition and early lactation periods were 3.1 and 3.2 for Control and 3.9 and 3.9 for the Supplemented groups, respectively. Dry matter intake (DMI) was recorded daily and feed sample was collected weekly. Cows were milked 3 times per day and daily yields were recorded. For seven weeks, milk samples were collected from each milking on 1 d per wk of each cow and composited for analysis of fat, protein, and lactose. Data were analyzed by using a mixed model (PROC MIXED, SAS Inst. Inc., Cary, NC) for a completely randomized design with repeated measures.

Results The results of DMI, milk production and components are present in Table 1. Dry matter intake was not significantly different between the diets in prepartum and postpartum. Inclusion of fish oil and canola oil in diet increased milk production in compare with the control diet ($p=0.042$). Milk fat percentage was significantly decreased in oil containing diet ($p=0.05$), but milk protein and lactose contents and also fat and protein yields were all similar among the dietary groups. Lactose production was significantly increased in the supplemented diet ($p=0.025$). Shingfield *et al.* (2006) has reported no effect on milk yield when they had used a combination of fish oil and extruded soybean in diet, but milk fat content and also milk fat and protein yields, and DMI were all decreased significantly by oil inclusion.

Table 1 Least squares means of production parameters in cows fed diets containing none (Control) or 2% oil (Supplemented) during first seven weeks of lactation

Parameter	Control	Supplemented	SEM	P
DMI, kgd ⁻¹				
Prepartum	13.78	14.47	0.33	0.171
postpartum	21.16	20.19	0.50	0.727
Milk, kgd ⁻¹	34.20 ^a	36.65 ^b	0.77	0.042
Fat				
%	3.12 ^a	2.76 ^b	0.77	0.005
Kgd ⁻¹	1.087	1.027	4.22	0.386
Protein				
%	2.86	2.83	0.035	0.587
Kgd ⁻¹	0.959	1.035	3.07	0.102
Lactose				
%	4.49	4.67	0.066	0.08
Kgd ⁻¹	1.51 ^a	1.70 ^b	5.66	0.025

Conclusion Results of this study showed that milk production increased by inclusion of oil in diet. Except than fat content and lactose yield, other parameters of milk composition were similar among the dietary groups. More studies needed to evaluate the effect of oil inclusion on lactation performance of early lactating cows.

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Effects of organic and inorganic zinc sources on production, composition and zinc concentration of milk in early lactating Holstein cows

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Introduction Zinc (Zn) is an essential nutrient for animals, functioning largely or entirely in enzyme systems and being involved in protein synthesis, carbohydrate metabolism, and many other biochemical reactions (Miller, 1970). This element is important in maintenance of health and integrity of epithelial tissue, such as skin (teats) and mammary tissue, because of its role in cell division and protein synthesis (Cook-Mills and Fraker, 1993). The most widely used products for zinc supplementation are zinc oxide, zinc sulphate and zinc methionine. Knowledge of organic Zn compounds is still incomplete. In particular, there is a lack of reports on the effect of such compounds in roughage based diets and only a limited number of studies have investigated the importance of Zn methionine in dairy cattle. Some research suggest that supplementing certain organic forms of zinc improve animal-production responses compared with those observed in ruminants that are supplemented with inorganic zinc (Spears, 1996). Therefore, the present study was conducted to compare organic and inorganic zinc supplements effects on production and components of milk. A second objective was to determine effects of different zinc sources on zinc concentration of milk.

Materials and methods Eighteen multiparous Holstein cows were randomly assigned in one of three dietary: 1) no supplemental Zn (control), 2) basal diet plus 500 mg of Zn/ kg of DM as ZnSO₄ .H₂O (ZnS) and 3) basal diet plus 500 mg of Zn/kg of DM as Zinc methionine (ZnM). In this experiment, dairy cows in first phase of lactation (35±3 days after parturition) were used. Milk samples were collected from each milking on 2 d per fortnight and composed for analysis of milk composition. Composed samples were analysed for fat, protein, lactose, solid not fat and somatic cell count (Micro Scan; FOSS Electric A/s, Denmark). Zinc concentration of milk in these samples analysed with atomic absorption (Chemtech, Analytical CTA-2000, UK). The data were analyzed using the mixed procedure of SAS (9.1) for a completely randomised design with repeated measures. Overall effect of treatment was tested using cow within treatment as the error term.

Results There was no significant difference between treatments in milk yield (Table1), however milk production in dairy cows that received ZnM were insignificantly increased (6.3%) than control group ($P < 0.05$). The milk protein, Solid Non Fat (SNF), lactose and fat percentages were not significant differed between treatments, but insignificantly milk protein percentage was higher than control group. Somatic cell count was reduced insignificantly with the elevated ZnM compared to control treatment (253.36 vs. 290.27 × 10³ cells/mL, respectively). Effects of Zn sources on Zn concentration in milk are shown in Figure 1. Zinc concentration in milk in the ZnM and ZnS groups were higher ($P < 0.05$) than control group (9.82 and 9.24 vs. 6.13, respectively), but there was not significant difference between ZnS and ZnM groups ($P > 0.05$) for this factor.

Table 1 Effects of Zn sources on production and composition of milk

Parameter	Control	ZnS	ZnM	SEM	P
Milk, kg/d	31.19	31.57	33.17	2.19	0.85
Fat %	3.73	3.80	3.72	0.11	0.82
Protein %	3.11	3.11	3.17	0.03	0.34
Lactose %	4.74	4.55	4.63	0.10	0.19
SNF %	8.59	8.40	8.47	0.10	0.46
SCC, 10 ³ cells/mL	253.36	290.27	136.63	77.02	0.14

Figure 1 Effects of Zn sources on Zn concentration in milk

Conclusions Data obtained from this study indicated that Zn supplementation with high concentration (500 mg/kg) had increased ($P < 0.05$) Zn concentration in milk. Incensement in milk production ($P > 0.05$) by supplementation of ZnM can be related to higher effect of organic Zn on biochemical process. Reduced insignificant SCC in dairy cows that received zinc treatments can be partially attributed to Zn role in immune function (Kellogg *et al.*, 2004).

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Linear and quadratic models as predictors of dry matter production and nutritive value of pastures located in the central plateau of Mexico

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Introduction Approximately 80% of all production costs are related to the purchase of forages and other feeds in dairy enterprises in Mexico. Resource poor, smallholder (*campesino*) systems are heavily reliant on the use of maize stover, non-cultivated/cultivated grass species and weeds to feed their dairy cattle, thus a nutritional understanding of local forage resources would be of considerable economic benefit. The objective of this study was to develop predictive models of herbage production and nutrient composition of four non-cultivated pastures (NcP), in order to minimise the cost of sample analysis and improve the utilisation of local forage resources in dairy enterprises.

Materials and methods Details about location of the NcP, their dry matter (DM) production, and crude protein (CP), neutral and acid detergent fibre (NDF and ADF), organic matter degradability (OMd) at 48 h, and metabolisable energy (ME) contents, number of samples and frequency of sampling are given in Rayas-Amor *et al.* (2009). Sward high (SH) measurements were recorded (n=500) once a month per pasture for 12 months from summer 2006 to end of spring 2007 using a rising plate meter in a “W” pattern across each pasture. Also, five SH measurements were completed within a 0.50 m² quadrant in each pasture and then all plants within the quadrant were cut with shears 5 cm above ground level, in order to estimate DM production. Linear and quadratic regression models were derived with Minitab[®] (v. 15), where two thirds of the data were used as a calibration set and one third used for model validation.

Results Only DMy, NDF and ADF were predicted from SH (P<0.05). Organic matter degradation and ME were predicted linearly from ADF in pasture 1 and 2, while in pasture 3 these values were predicted from NDF and had a higher R² of prediction. The prediction of CP was higher with a quadratic model in all pastures and was directly predicted from SH in pasture 3 while in pasture 1 and 2 this nutrient had higher R² coefficient using ADF as the predictor. The prediction interval in Table 1 shows where the predicted value is expected to fall. It has two values that indicate how big the interval is in the lower and upper limit; the bigger the values are, the bigger the intervals are in each limit, therefore the standard error of the prediction is bigger. Only DMy in Pastures 1 and 3 had a wider interval in the upper limit.

Table 1 Predictive models for DM (kg/ha); NDF, ADF and CP (g/kg DM); OMd (g/kg OM) and ME (MJ/kg DM)

	P	Par	Model	R ²	P-value	Prediction Interval 95%	
						Lower limit	Upper limit
Multi-species	1	DMy	151.0 + 272.0*SH	0.782	***	(500, 400)	(4500, 2500)
		NDF	828 - 6.76*SH	0.396	*	(750, 700)	(840, 810)
		ADF	372 - 2.79*SH	0.334	*	(330, 310)	(390, 375)
		CP	- 3242 + 20.36 ADF - 0.03089 ADF ²	0.559	*	(60, 30)	(150, 120)
		OMd	1635 - 3.43*ADF	0.531	**	(460, 320)	(600, 450)
		ME	23.7 - 0.0497*ADF	0.473	**	(6.5, 4.5)	(8.5, 6.5)
Multi-species	2	DMy	- 151.0 + 280.5* SH	0.967	***	(400, 200)	(850, 700)
		NDF	862 - 47.7*SH	0.391	*	(680, 600)	(880, 840)
		ADF	411 - 26*SH	0.437	*	(300, 260)	(430, 400)
		CP	- 24.6 + 1.080*ADF - 0.002244*ADF ²	0.774	***	(80, 45)	(120, 80)
		OMd	1088 - 1.67*ADF	0.916	***	(550, 425)	(625, 500)
		ME	16 - 0.0245*ADF	0.904	***	(8.0, 6.2)	(9.4, 7.2)
Mono species	3	DMy	23.77 + 211.0*SH	0.963	**	(220, 100)	(1400, 500)
		NDF	486 + 16.8 *SH	0.397	*	(700, 610)	(450, 410)
		CP	52.5 + 98.09*SH - 12.06*SH ²	0.467	*	(60, 49)	(370, 320)
		OMd	1203 - 0.99*NDF	0.700	**	(650, 560)	(775, 700)
		ME	17.6 - 0.014*NDF	0.700	**	(10, 8.5)	(11.5, 10.5)

P pastures; Par Parameter; DM dry matter yield, NDF neutral detergent fibre, ADF acid detergent fibre, CP crude protein, OMd organic matter degradation, ME metabolisable energy; R² of prediction; * (P<0.05), ** (P<0.01), *** (P<0.001)

Conclusions Sward height showed potential as a method for predicting DMy, NDF, ADF and CP contents. Although OMd and ME concentration were not estimated directly from SH, they could be estimated from ADF or NDF. Further validation of these models is required.

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Effect of substitution barley grain with dried sugar beet pulp on ruminal pH and ammonia nitrogen concentration of Holstein steers

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Introduction Grains are often substituted for forage in ruminant diets to increase feed intake, diet fermentability, and animal growth rate. However, greater dietary starch concentration can lead to digestive disorders, such as ruminal acidosis and reduce performance (Owens *et al.*, 1998). Adding non-forage fibre source, such as sugar beet pulp (SBP), to low-forage diets might reduce the negative effects of increased starch fermentation without increasing the filling effect of the diet to the same extent as forage fibre. So, substituting SBP for barley grain in a diet with moderately low forage content should alter ruminal fermentation and might increase mean or minimum ruminal pH. Thus, the objective of this study was to evaluate the effect of the substitution of barley grain with SBP on ruminal pH and ammonia nitrogen (NH₃-N) concentration in Holstein steers.

Materials and methods Four Holstein steers with initial body weight of 368 ± 8 kg fitted with ruminal fistulae were used in a 4×4 Latin square design (28 days of each period). Basal experimental diet consisted of 15% maize silage, 20% lucerne hay, 33% barley grain, 17% soyabean meal, 13.8% wheat bran, 0.5% calcium carbonate, 0.2% salt, and 0.5% mineral and vitamin premix on dry matter basis. Barley grain was substituted with SBP as 0.0%, 33%, 66% or 100% (SBP0, SBP33, SBP66, and SBP100, respectively). Steers were housed in individual pens, and fed 9.5 kg of diet DM as total mixed ration twice daily at 0800 and 1600 h. At day 28 of each experimental period, rumen fluid samples were collected, from fistulae by suction, before the morning feeding and 1, 2, 3, 4, 6 and 8 hours post feeding. Sample pH was immediately recorded using a portable pH meter (Metrohm 744). For samples designated for NH₃-N analysis, 5 ml of ruminal fluid from each collection were acidified with 5 ml of 0.2 N HCl. Samples were analysed for NH₃-N concentration using distillation method (Kjeltec 1030 Analyzer tecator). Data were analysed using the GLM procedure of SAS ($Y = \text{Mean} + \text{Treatment} + \text{Animal} + \text{Period} + \text{Time} + \text{Time} \times \text{Treatment} + \text{Residual}$), while including the sampling time as a repeated measurement.

Results The ruminal pH and NH₃-N concentration at different sampling time are shown in the Table 1. Results indicated that both ruminal pH and NH₃-N concentration were significantly influenced by the treatments and sampling time. Rumen pH of the animals fed SBP0 and SBP33 was minimum and maximum respectively, but similar for SBP66 and SBP100. In contrast rumen NH₃-N concentration decreased by inclusion of SBP in the diets ($P < 0.05$). Ruminal concentration of NH₃-N for animals fed SBP0 was maximum, but similar for SBP33, SBP66, and SBP100.

Table 1 Ruminal pH, and ammonia-N concentration (mg/dl) of steers fed diets containing different levels of sugar beet pulp

Items	Time(h)	Treatments [†]				Treatment effect		Time effect	
		SBP0	SBP33	SBP66	SBP100	s.e.m.	P	s.e.m.	P
pH	0.0	6.76	6.93	6.72	6.76	0.107	**	0.117	**
	1.0	6.23	6.22	6.22	6.24				
	2.0	6.17	6.28	6.12	6.29				
	3.0	6.08	6.43	6.21	6.31				
	4.0	5.98	6.51	6.35	6.34				
	6.0	6.22	6.50	6.37	6.36				
	8.0	6.32	6.60	6.40	6.50				
NH ₃ -N	0.0	22.9	18.73	19.85	18.58	0.73	**	1.56	**
	1.0	32.52	31.95	30.56	30.21				
	2.0	36.42	33.78	34.28	31.59				
	3.0	37.35	31.87	32.08	29.86				
	4.0	37.75	28.65	26.43	26.93				
	6.0	26.87	22.08	21.14	18.48				
	8.0	24.76	18.43	19.29	14.8				

[†] Barley grain was substituted with SBP as 0.0%(SBP0), 33%(SBP33), 66%(SBP66) or 100%(SBP100).

** $P \leq 0.01$.

Conclusions Results of the present study demonstrated that the increasing inclusion of SBP in the diets caused an increase in rumen pH and decrease NH₃-N concentration. Rumen pH of the steers fed basal diet (SBP0) was markedly decreased during 2 to 4 hours after morning feeding. Marounek *et al.* (1985) reported that unlike starch, pectin fermentation produces very little lactate as pectinolytic bacteria are inhibited at low pH. Decreasing of rumen NH₃-N concentration with inclusion of SBP might be due either to a slower release of ammonia from the diet or more rapid uptake by microorganisms in the presence of a fermentable source of carbohydrate, such as pectin. Overall, the results of the present experiment suggested that substitution of barley grain with SBP had a significant effect on rumen synchronising as observed in increased pH and decreased ammonia-N concentration.

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Chewing behaviour of Holstein steers fed diets containing different levels of non-forage fibre in a low forage diet

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Introduction Sugar beet pulp (SBP) is fed to ruminants as a non-forage fibre source (NFFS) ingredient. Early work by Ronning and Bath (1962) demonstrated that SBP was similar in feeding value to barley grain for lactating dairy cattle, supporting classification of beet pulp as an energy concentrate. Sugar beet pulp contains approximately 40% neutral detergent fibre (NDF) and is unique in its high concentration of neutral detergent soluble fiber, especially pectic substances (~25% of dry matter (DM)). However, the effects of SBP when substituted with different feed sources in ration are variable that depend on chemical composition, types and physical characteristics. The time which cows spent eating and ruminating (total chewing time) is a measure of the physically effective fibre value of a feed. The objective of the present experiment was to evaluate the effect of substitution of barley grain with SBP as a NFFS on chewing activity of Holstein steers.

Materials and methods Four Holstein steers with initial body weight of 368 ± 8 kg were used in a 4×4 Latin square design (28 days of each period). Basal experimental diet consisted of 15% maize silage, 20% lucerne hay, 33% barley grain, 17% soyabean meal, 13.8% wheat bran, 0.5% calcium carbonate, 0.2% salt, and 0.5% mineral and vitamin premix on dry matter basis. Barley grain was substituted with SBP as 0.0%, 33%, 66% or 100% named SBP0, SBP33, SBP66, and SBP100, respectively. Steers were housed in individual pens, and fed 9.5 kg of diet DM as total mixed ration twice daily at 0800 and 1600 h. Eating and ruminating activities of individual cows were visually observed and recorded at 5 min intervals for 24 hours on day 27 of each period. Feed samples were analysed for NDF according to Van Soest *et al.*, (1991) and NDF was expressed as the ash free residue after extraction with boiling neutral solutions of sodium lauryl sulphate and EDTA. Data were analysed using the mixed model procedure of SAS (2001) ($Y = \text{Mean} + \text{Treatment} + \text{Animal} + \text{Period} + \text{Residual}$) and the means compared by the Duncan test ($P < 0.05$).

Results Substitution of SBP increased physically effective NDF (peNDF) of rations from 22.77 to 28.71 % DM ($P < 0.05$, Table 1). No differences ($P > 0.05$) were observed between treatments for eating time per day, per kilogram of DM intake (DMI) and per kilogram of NDF intake ($P > 0.05$, Table 1). As diet peNDF was increased, with added SBP, ruminating activity as according to all the evaluated indices was increased ($P < 0.05$), but similar for SBP0 and SBP33. Similarly, time spent in total chewing (eating + ruminating) as according to all the evaluated indices was increased with inclusion of SBP in treatments, but similar for SBP0 and SBP33.

Table 1 chewing behaviour of steers fed diets containing different levels of sugar beet pulp as non-forage fibre source

Items	Treatments [†]				s.e.m.	P-value
	SBP0	SBP33	SBP66	SBP100		
peNDF [‡] , %	22.77 ^c	24.15 ^c	26.25 ^b	28.71 ^a	0.36	**
Eating time, min						
per day	111.2	123.7	128.8	143.7	23.9	ns
per Kg of DMI	12.2	13.6	14.1	15.7	2.6	ns
per Kg of NDF intake	37.9	39.9	40.1	42.7	7.6	ns
Ruminating time, min						
per day	232.5 ^b	267.5 ^b	365 ^a	391.2 ^a	41.3	**
per Kg of DMI	25.5 ^b	29.3 ^b	40 ^a	42.9 ^a	4.5	**
per Kg of NDF intake	79.3 ^b	86.8 ^b	113.3 ^a	116.4 ^a	13.06	**
Total chewing time, min						
per day	343.7 ^b	391.2 ^b	493.7 ^a	535 ^a	40.1	**
per Kg of DMI	37.7 ^b	42.9 ^b	54.1 ^a	58.7 ^a	4.4	**
per Kg of NDF intake	117.3 ^b	126.9 ^b	153.3 ^a	159.2 ^a	12.6	**

[†] Barley grain was substituted with SBP as 0.0%(SBP0), 33%(SBP33), 66%(SBP66) or 100%(SBP100).

[‡] PeNDF = Ration NDF multiplied by amount of DM >1.18 mm (Kononoff *et al.* 2002).

a, b, c Means in row with different superscript letter are different, ** $P \leq 0.01$.

Conclusions It was concluded that inclusion of SBP had a positive effect on ruminating and total chewing time as reported per day, per kilogram of DMI and per kilogram of NDF intake. Results of previous study demonstrated no effect of SBP substitution for maize grain on time eating per day or per kilogram of DMI (Clark and Armentano, 1997). Taken together, results of this study suggest that SBP might be a source of physically effective fibre by the definition of increasing chewing time.

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The effect of feeding ensiled alternative forages compared with ensiled ryegrass on excreta losses from growing lambs

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Introduction An experiment investigated the effects of using ensiled alternative forages compared with ensiled ryegrass on lamb productivity, nutrient use efficiency and total excreta losses in a lamb finishing system. Lambs offered alternative forages had a higher liveweight gain and nitrogen use efficiency than lambs offered ryegrass silage ($P < 0.001$) (Marley *et al.*, 2007). However, balancing the input and output of nutrients within the farm system is critical to ensure both short-term productivity and long-term sustainability, as producers aim to establish whole-farm nutrient balance plans and reduce reliance on bought-in fertilisers. Here we present the findings of the effects of feeding ensiled red clover (*Trifolium pratense*), lucerne (*Medicago sativa*), pea (*Pisum sativum*), kale (*Brassica oleracea*) compared with ensiled hybrid ryegrass (*Lolium hybridum*) on excreta losses from these growing lambs and, therefore, the potential impact of incorporating these forages on nutrient budgets within livestock systems.

Materials and methods Forages were established on soils of the Rheidol series (52° 27'N, 4° 01'W), harvested and ensiled as previously described in Marley *et al.* (2007). One hundred Suffolk-cross lambs (approx. 8 months) were housed as one group and offered a single ryegrass silage *ad libitum* for 6 weeks. Lambs were then grouped within gender according to live weight (mean 30.9, s. d. 2.29) and body condition score (mean 2.3, s.d. 0.154) resulting in four blocking groups, nominally large castrates, large females, small castrates and small females. Within each blocking group, five lambs were allocated at random to each forage treatment. The five treatment groups were segregated and experimental silages were introduced gradually into the diet over a 7-d period. A further 7-d period with *ad libitum* access to their allocated silage was allowed for dietary adaptation. All lambs were then transferred to a sheep-housing facility that was arbitrarily divided into four blocks of five pens. Each blocking group was allocated at random to a set of five adjacent pens and silage treatment groups were allocated at random to pens within this. After careful monitoring, lambs on the pea silage were found to have low liveweight gains and, so as to ensure animal welfare was not compromised, this treatment was discontinued. Mesh flooring placed over plastic trays were used in one of the four blocks and lambs were rotationally moved every 14 d, in their respective blocks, so that faeces and urine were collected from beneath all lambs during an 8 week period. The total amounts of faeces and urine excreted were determined every 14 d and sub-samples were collected, mixed thoroughly, diluted with a known amount of water and sub-sampled for analysis for N and ammonium-N concentrations. Within-pen means of excreta losses were subjected to ANOVA assuming a randomised block design and treating blocking group as a random effect. Treatment means were compared using the Student-Newman-Keuls test, using Genstat®, Version 8.1 (Payne *et al.*, 2005).

Results Although lambs fed on kale silage produced a higher amount of excreta than lambs on other silages ($P < 0.001$), the total dry matter (DM) losses from lambs fed on kale silage were lower than lambs fed the legume silages, confirming visual observations that lambs on kale silage produced more urine than those on other treatments (Table 1). In terms of DM output, lambs fed red clover or lucerne silage produced more excreta than lambs on either ryegrass or kale silage ($P < 0.01$). Total nitrogen (N) excretion was higher from lucerne silage than from red clover silage ($P < 0.05$) and both legume silages showed higher rates of N excretion than either ryegrass or kale ($P < 0.001$). In absolute terms, lambs fed lucerne excreted the most ammonium-N ($P < 0.05$). The proportion of nitrogen in the form of ammonium in slurry from lambs fed ryegrass silage was significantly lower ($P < 0.05$) than from lambs fed lucerne, red clover or kale silages.

Table 1 Excreta losses over 14d from groups of five lambs offered different silages.

	Ryegrass	Red clover	Lucerne	Kale	s.e.d.	P*
Total excreta (kg)	65 ^c	99 ^b	118 ^b	191 ^a	11.9	< 0.001
Dry matter (kg)	16.8 ^b	30.5 ^a	33.7 ^a	16.6 ^b	2.99	< 0.001
Total Nitrogen (TN) (g)	708 ^c	1459 ^b	1995 ^a	769 ^c	120.6	< 0.001
Ammonium-N (g)	59 ^d	322 ^b	516 ^a	166 ^c	35.7	< 0.001
(g/kg TN)	89 ^b	225 ^a	259 ^a	221 ^a	19.8	< 0.001

* , with 9df for error. Means within rows with different superscripts differ significantly ($P < 0.05$).

Conclusion Feeding alternative ensiled forages to lambs significantly altered their excreta losses compared with feeding ensiled ryegrass, with significantly more nitrogen being excreted when lambs were offered lucerne silage compared to the other treatments. These findings demonstrate that alternative forages can have a profound economic and environmental impact on nutrient budgets within livestock systems, with the nitrogen in excreta representing either a substantial gain or loss of nutrients to the whole system if it is not correctly stored and recycled.

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Effects of feeding pistachio hull and interaruminal infusion of urea on feed intake, ruminal and abomasum N-NH₃ and blood metabolites in Iranian Balochi sheep

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Introduction Due to binding tannin with protein and cellulose, it was hypothesized that pistachio hull tannin can decrease the digestibility of protein and cellulose. Pistachio Hull (PH) is the main pistachio by-product produced from the pistachio dehulling process. PH contained 12.7, 5.7, 16.6, 25, and 20% ash, EE, CP, NDF, and ADF, respectively. The nutritional responses to high levels of dried pistachio by-product in the diet have not been clearly researched. The main objective of this experiment was to evaluate the effects of feeding pistachio Hull at high levels on feed intake, ruminal pH, N-NH₃ and blood metabolites in Balochi sheep.

Materials and methods Three rams (39 ±2Kg, aged 20 to 24) were assigned into a replicated 3×3 Latin square design with 3-wk periods. The dietary treatments were on follows: 1) the control diet, 2 and 3) the control diet plus 20 and 40% of beet pulp which were replaced with dried pistachio hull (W/W). Three rams surgically equipped with ruminal cannulas and "T" type cannulas in the abomasum were randomly allocated to the treatments. Urea was continuously infused into the rumen via peristaltic pump. Urea was diluted with water and infused at the rate of 1 mL/min to provide 17 g of urea/d. Feed intake recorded daily and ruminal samples were taken from each ram 4 h after feeding and subsequently, 50 mL of ruminal fluid (strained through two layers of cheesecloth) was acidified with 10 mL of HCl solution (50%, vol/vol) and stored frozen for ammonia N analysis. Abomasum samples were taken simultaneously from all rams twice daily during a period of four successive days. Blood samples were drawn from the jugular vein into vacuum tubes on the last days of each experiment period at about 3-4 h post feeding. Collected serum was frozen in -20°C after centrifuging until future analysis for blood metabolites. Data analysed with SAS (9.01).

Results The dry matter intake (DMI), ruminal and abomasum N-NH₃ and blood metabolites are presented in the Table 1. Differences between treatments for DMI, N-NH₃, glucose, cholesterol and triglyceride were statistically significant ($P<0.05$) but there were not affect on creatinine, K and Na concentration ($P>0.05$). The substitution of dried pistachio by product by beet pulp at 20 and 40 % levels decreased feed intake, ruminal N-NH₃, glucose, cholesterol and triglyceride. But increased abomasums N-NH₃ and BUN compare to the control diet. This result is in agreement with the other results (Naserian and Vahmani, 2002; Ben Salem *et al.*, 2005).

Table 1 Dry matter intake, ruminal PH and ammonia N concentrations of blochi sheep.

Item	Treatments (dried pistachio by product levels %)			SEM
	0	20	40	
DMI (kg day ⁻¹)	0.744 ^a	0.744 ^a	0.653 ^b	1.38
Ruminal ammonia N(mg/dl) Time after feeding, 4	36.14 ^a	33.41 ^a	26.14 ^b	0.83
Abomasum N-NH ₃ (mg/dl)	25.21 ^b	26.09 ^b	31.9 ^a	1.03
Blood metabolites (mg/dl)				
Glucose	64 ^a	62 ^a	58 ^b	1.66
BUN	13.66	15.33	17.66	0.98
Cholesterol	35.33 ^{ab}	40 ^a	31 ^b	0.93
Triglyceride	13 ^a	12 ^a	8.3 ^b	0.64
Creatinine	0.9	0.85	1	0.004
K (Potassium)	4.8	4.9	5.2	0.04
Na (Sodium)	151.5	153	155	0.11

Conclusion The obtained results indicated that feeding dried pistachio by product at high levels led to decrease feed intake, ruminal ammonia N concentration, blood glucose, cholesterol, triglyceride and increase abomasums N-NH₃ and blood BUN. The addition of dried pistachio by product at 20% level (dry matter basis) to the balochi sheep ration will decrease the cost of ration without any side effects on animal performance. This by-product can be regarded as a source of effective fiber for ruminant animal. However, more experiments are needed for conformation of these finding.

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Effect of inoculants on fermentation and nutritive value of unwilted and wilted Lucerne silage

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Introduction Homolactic bacteria have been used to inoculate silages and improve their fermentation. Inoculation with these microbes has increased the rate and extends of lactic acid production in silage and decreased proteolysis and production of volatile organic acid. Wilting of high moisture forages prior to ensiling usually enhances preservation and intake responses of cattle fed silage. Wilting creates an ensiling environment that is conducive to a restricted proteolysis and fermentation, and the resulting silage is typically higher in water soluble carbohydrate (WSC) and lower in fermentation acids. The microorganisms used in silage inoculants depend on a high level of moisture for growth. Pahlow and Weissbach (1996) reported that only 10% of the total population of epiphytic lactic acid bacteria (LAB) on grasses grew on modified, selective agar with an water activity (aw) of 0.952 (forage with a DM content of 50%), suggesting that epiphytic LAB were not very osmotolerant. However, Lucerne is often wilted to concentrations above 30% DM before ensiling to reduce the chances of clostridial fermentation and In these silages, it may be speculated that use of osmointolerant inoculants can include two Excellences of wilting and inoculants to make better silage. The aim of the present study was to assess the effects of wilting and inoculants on Lucerne silage quality.

Materials and methods Silage was prepare from fourth-cut Lucerne forage, harvested in stage of prebud and 22 % of DM and divide to two parts. Part 1 was chapped and ensiled 1; without treatment, 2; treated with LALSIL inoculants, comprise of *Lactobacillus plantarum* and *Propionibacterium acidipropionici* that were applied at 1.0×10^6 CFU g⁻¹, part 2 was farm wilted to 37% DM, chapped and ensiled 3; without additive and 4; treated with LALSIL. The silage was allowed to ferment at ambient temperature for 90 days and sub-sampled. Three composite samples of each treatment were analyzed for the variables in Table 1 by methods of AOAC. *In situ* dry mater degradability was meseared with method of Vanzant *et al* (1998), two ruminally fistulated (40 kg) ghezel sheep fed Lucerne hay twice daily (0900 and 1600 h) were used for incubation of samples. Six replication were used for each treatment .samples were incubated for 24 h. The data was statistically analyzed using ANOVA in proc GLM.

Table 1 Silage quality of wilted and unwilted Lucerne silage treated with inoculants

Treatments		1	2	3	4	SEM
Chemical composition	pH,	4.56 ^c	4.77 ^b	5.02 ^a	4.63 ^c	.03
	DM	20.17 ^b	20.95 ^b	37.57 ^a	38.19 ^a	.44
	CP, %DM	22.52 ^b	22.15 ^b	2.12 ^b	23.92 ^a	.33
	NDF, %DM	29.81 ^{ab}	31.54 ^a	30.30 ^a	26.50 ^b	1.05
	Acetic acid, %DM	3.05 ^a	2.67 ^a	1.41 ^b	.83 ^c	.21
	Lactic acid, %DM	9.90 ^a	9.85 ^a	5.83 ^b	5.66 ^b	.06
	La:ac ¹	3.24 ^c	3.70 ^{bc}	4.16 ^b	6.75 ^a	.26
	N(NH ₃) ² , %DM	1.23 ^a	1.32 ^a	.79 ^b	.58 ^c	.05
	ADIN, ³ %DM	.40 ^b	.43 ^a	.39 ^b	.33 ^c	.01
	<i>In situ</i> DMD ⁴ , %DM	80.64 ^b	81.22 ^b	80.49 ^b	87.02 ^a	.83

Means on the same line followed with the same superscript or no superscript, do not differ significantly (p<0.05).

Treatment 1= unwilted, treatment 2 = unwilted + inoculant, treatment 3= wilted, treatment 4 = wilted + inoculant,

¹ lactic acid :acetic acid, ² ammonia N, ³ acid detergent insoluble nitrogen, ⁴ in-situ DM degradability

Result The composition of the silages in percentage of DM is presented in table 1. Wilting increased silage pH, DM and lactic acid: acetic acid and decreased silage acid detergent insoluble nitrogen (ADIN), ammonia N, acetic acid and lactic acid (p<0.05). Inoculant increased silage pH, ADIN and N (NH₃) in unwilted but decreased effectively in wilted silage (p<0.05). Acetic acid decrease and la: ac and in-situ DM degradability (*In situ* DMD) increased only in wilted silage. In this reaserch prop ionic acid concentration was in undetectable level, because *Propionibacteria* propagation inhibits in low pH and also microbes may convert propionic acid to other organic acid in silage.

Conclusion this result revealed that LALSIL had better performance with lower pH and proteolysis in high DM and supported our hypothesis that high DM can inhibit growth of hetrofermentative bacteria and entrobacteria but can not drastically affect homofermentative lactic acid bacteria.

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An on-farm evaluation of the effects of season of shearing on ewe and subsequent lamb performance

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Introduction The weight of lamb carcass produced per hectare is one of the major factors influencing the efficiency of lamb production and is influenced by the litter size and subsequent lamb growth rate (Keady and Hanrahan, 2006). On many sheep farms ewes are housed during the winter to facilitate increased stock carrying capacity. Ewes are normally shorn once yearly, usually in early summer. Previous studies at Athenry (Keady *et al.*, 2007, Keady and Hanrahan, 2008, 2009) have shown that shearing ewes at housing increased subsequent lamb birth and weaning weights by up to 0.6 and 2.5 kg, respectively. Therefore as a consequence of reducing age at slaughter by approximately 2 weeks, whilst increasing price received per kg of carcass. However, shearing at housing may increase management inputs as ewes are normally housed in smaller groups and need to be dry prior to shearing. Shearing in the autumn, prior to mating, enables the flock to be assembled under more favourable conditions. A previous study at this centre (Keady and Hanrahan, 2008) showed that shearing prior to mating tended to increase subsequent lamb birth weight (+0.3 kg) but did not alter litter size or lamb viability relative to lambs born from ewes which were shorn in late May. The aim of this study was to further evaluate the effects of season of shearing on fertility of March - lambing ewes and on subsequent lamb performance.

Materials and methods The study was undertaken on a commercial farm. A total of 353 ewes [(Belclare cross) (initial LW = 59.8 kg, initial CS = 3.5)] which had been allocated at random in September 2006 to one of three shearing treatments as follows: shorn prior to mating, at housing and conventional shorn (September 2006, December 2006 and June 2007, respectively). Subsequently the ewes on the prior to mating, at housing and shorn treatments were shorn 7 September 2007, 20 December 2007 and 13 June 2008, consequently all ewes had been shorn 12 months previously. All ewes were mated at a natural oestrus, as one flock, to a panel of 5 Charollais rams which joined with the ewes on 21 October 2007. The ewes were housed on 15 December and offered hay until mid January. Subsequently the ewes were grouped according to litter size (ultrasonic scanning) and received a straw based diet supplemented with concentrate. For weeks 8-7, 6-5, 4-3 and 2-lambing ewes carrying singles received 0.7, 0.8, 0.9 and 1.0 kg concentrate daily, twins received 0.8, 1.1, 1.3 and 1.4 kg concentrate daily and triplets/quads received 1.0, 1.2, 1.5 and 1.5 kg concentrate daily respectively. Ewes were weighed and condition scored at shearing in September, December and June and at 10 and 14 weeks post lambing. Fleeces were weighed at shearing. Lambs were weighed at birth and 10 and 14 weeks post lambing. The data were analysed using Proc GLM and Proc MIXED of SAS as appropriate.

Results The effects of shearing treatment on ewe and subsequent progeny performance are presented in Table 1. Ewes shorn at housing had heavier fleeces than ewes shorn in June or September. Season of shearing did not alter ($P>0.05$) ewe liveweight or condition score at housing or weaning, or on litter size or number reared. Shearing at housing tended ($P>0.05$) to increase lamb birth and weaning weights.

Table 1 The effects of season of shearing on ewe and progeny performance

		Treatment			s.e.	Sig.
		June	Sept	Dec		
Ewe weight (kg)	- housing	69.0	68.9	70.9	0.75	NS
	- weaning	68.0	68.5	70.3	0.89	NS
Ewe CS	- housing	2.9	3.0	2.9	0.03	NS
	- weaning	3.0	2.9	3.0	0.05	NS
Fleece weight (kg)		2.76 ^a	2.56 ^a	3.05 ^b	0.061	***
Fertility		0.96 ^b	0.90 ^a	0.89 ^a		*
Litter size		1.97	2.04	1.99	0.065	NS
Number reared / ewe lambing		1.52	1.64	1.48	0.078	NS
Lamb weight (kg)	- birth	4.21	4.35	4.45	0.094	NS
	- weaning	28.2	28.2	29.2	0.570	NS
Lamb gain birth – weaning (g/d)		234	235	243	5.46	NS

Conclusions Whilst the performance of the lambs from birth to weaning in the current on-farm study was moderate, ewes which had been shorn at housing produced heavier fleeces and lambs which tended to be heavier at weaning. June shorn ewes were most fertile.

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Effects of different levels replacement of ensiled pistachio epicarp with corn silage on fattening parameters and lamb performance

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Introduction There is about 430000 hectares of pistachio garden in Iran and annual dry pistachio production is 688000 tones. There are some problems with this by-product are including: 1) deterioration in less than 24 hours so it could not stored for long term. 2) Pistachio epicarp contains high level of tannins and other phenolic compounds, and there is little information about its effects in ruminants. 3) This by-product is potentially an environmental pollutant and cost effective for disposal. A study was conducted to investigate possibility of different levels replacement of ensiled pistachio epicarp (EPE) with corn silage and its effects on growth rate and carcass composition of lambs.

Materials and methods To investigate nutritive value and the possibility of using EPE in animal feeding and determination of optimum level in diet, four groups of ten male lambs (age 6 months and average weight 35.23±0.66 Kg equal in initial weight, each lamb were housed in an individual box), in a completely randomized design (4×10) were fed for 90 days, iso-caloric, iso-nitrogenous, iso-RDP (rumen degradable protein), iso-UDP (rumen undegradable protein, iso-forage, iso-Ca:P ratio and iso-NDF (neutral detergent fibre) diets, containing either no added ensiled pistachio epicarp (control) and added levels 8.7, 17.33 and 26 percentage ensiled pistachio epicarp in rations (replacement ratio was 0, 33.33, 66.66 and 100 percentage of metabolizable energy). After 90 days feeding, the lambs were slaughtered and carcass parameters were measured (Marinova *et al.*, 2001). All kidneys and livers were fixed in %10 formalin and used for histology and pathology tests. Data were analysed with SAS software in proc ANOVA.

Results EPE included: %9.79 total phenolic compounds, %4.73 total tannins and %1.92 condensed tannins. As ensiled pistachio epicarp (EPE) level increased in rations instead of maize silage, feed intake (FI) decreased and feed conversion ratio (FCR) increased. The weight of empty digestive tract were equal (ns) in treatments so groups were similar in digestive tract capacity and higher feed intake that observed in treatment 1 and 2 were due to 1) Higher digestibility of rations due to no or lower phenolic compounds. 2) Higher digestibility (optimum retention time) because high level of tannins in rations result to higher consuming water. 3) Better taste (phenolic compounds are not palatable). And increase in FCR because: 1) Higher heat increment (see Table 1, hypertrophy in liver due to detoxification blood of tannins especially hydrolysable tannins), 2) As tannins could binding to feedstuffs protein and by-pass them to intestine, a low level of tannin like treatment two can supply an approximate by-pass protein for animals but the higher levels of tannin results to high amount of UDP and low amount of RDP in animals. 3) Tannins can increase CCK hormone that cause fullness feeling in animals. 4) Tannins can bind sulphurs and decrease its bioavailability and this mineral is an essential mineral for rumen micro organism's growth that can affect animal growth (Brody, 1945, Makkar *et al.*, 1997a) Microscopic and macroscopic pathology of kidneys and livers did not show any damage in all treatments. Kidney and bladder stones were not observed in any treatments.

Table 1- Mean and standard error of performance and some carcass parameters

	FI	FCR	ADG	F.W	FI/Kg.MW	Liver (%)
T1(0%EPE)	191.17±3.09 ^a	8.54±0.87 ^a	248.61±4.58 ^a	57.48±0.81 ^a	110.34±1.09 ^a	1.82±0.03 ^a
T2(8.7% EPE)	189.85±3.57 ^a	8.57±0.60 ^a	246.11±4.18 ^a	57.30±0.79 ^a	109.83±1.70 ^a	1.87±0.03 ^b
T3(17.33%EPE)	175.09±3.13 ^b	8.89±0.95 ^b	218.89±3.66 ^b	54.95±0.63 ^b	104.44±2.18 ^b	1.94±0.03 ^c
T4(26% EPE)	166.90±2.96 ^c	9.12±0.12 ^c	203.33±4.86 ^c	53.70±0.64 ^c	101.25±2.20 ^c	1.99±0.05 ^d

FI(feed intake,gr/day), FCR(feed conversion ratio), ADG(average daily gain gr/day), FW(final weight, Kg), FI/Kg.MW= feed intake/ Kg metabolic weight)

Table 2 Mean and standard error of performance and some carcass parameters

	Kidney (%)	E.S	F.S	E.I	F.I	I.F ¹	Fat. tail
T1(0%EPE)	0.33±0.03 ^a	3.11±0.12 ^a	13.41±0.16 ^a	4.55±0.10 ^a	7.70±0.09 ^a	0.41±0.03 ^a	19.20±0.63 ^a
T2(8.7% EPE)	0.29±0.03 ^b	3.11±0.16 ^a	13.37±0.14 ^a	4.50±0.14	7.82±0.08 ^{ab}	0.38±0.03 ^a	19.20±0.10 ^a
T3(17.33%EPE)	0.29±0.02 ^b	3.20±0.07 ^a	13.33±0.14 ^a	4.60±0.14 ^a	7.78±0.15 ^{ab}	0.41±0.03 ^a	19.22±0.57 ^a
T4(26% EPE)	0.32±0.02 ^b	3.20±0.10 ^a	13.63±0.15 ^b	4.59±0.19 ^a	7.90±0.16 ^b	0.40±0.02 ^a	19.04±0.71 ^a

FS(%full stomach), ES(empty stomach), F.I(%full intestines), E.I(%empty intestines), Kid(Kidneys) , IF(internal fat),(1.ratio to cold carcass)

Conclusion The results showed that feeding lambs with ensiled pistachio epicarp up to 8.7 percentage of total dry matter intake did not affect lambs performance, feed conversion, body composition and health of animals.

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Effect of cobalt supplementation on performance of Mehraban lambs

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Introduction A major physiological effect of cobalt deficiency is loss of appetite (Smith, 1997). Rumen micro-organisms require cobalt for the synthesis of Vitamin B₁₂, which acts as a cofactor for protein and energy metabolism enzymes, namely methylmalonyl coenzyme A mutase and methionine synthase (Kennedy et al. 1992). Cobalt deficiency, therefore, impairs the energy and protein metabolism and thus growth and development of the deficient animal, which can be defined as changes in the weight, shape and size of the body. Sheep tend to be extremely susceptible to Co deficiency and develop a normocytic and normochromic anaemia, anorexia, reduced weight gains and photosensitivity (Vellema *et al.*, 1996). The present study was designed to evaluate the effects of dietary Co level on performance of Mehraban male lambs.

Materials and methods Twenty eight, 8-9-week-old lambs were housed in individual pens and fed with the basal diet containing 0.0884 mg Co/kg DM. After 2 weeks adjustment to the experimental feeding system, lambs were weighed and randomly assigned to 4 treatments with 7 lambs in each: control group was fed with the basal diet, and 3 supplemented groups were fed the basal diet and supplied with 0.25, 0.50 and 1.00 mg Co/kg DM as reagent grade CoSO₄·7H₂O for 10 weeks. Basal diet was formulated to meet all nutrient requirements with the exception of Co for lambs (NRC, 1985). Daily feed offerings and refusals were measured to obtain net feed intake for each animal. Lambs were weighed before morning feeding and initial and final BW were the average of BW obtained on two consecutive days. Cobalt was measured with an atomic absorption spectrophotometer (PHILIPS Model PU9100, single beam) according to the procedures of AOAC (1990).

Results Average daily gain (ADG) and Feed Conversion Ratio (FCR) were highest at 0.5 mg Co/kg DM supplementation. (Table 1). Cobalt supplementation increased ($P < 0.05$) average daily feed intake (ADFI) over the total study.

Table 1 Effects of dietary Co supplementation on performance¹ lambs (DM basis)

	Co supplemental levels (mg/kg)				SEM ³
	0	0.25	0.5	1	
Initial BW (kg)	37.2	37.0	37.1	37.4	0.89
Final BW (kg)	49.53	51.17	52.21	51.18	1.01
ADG (g)	176.33 ^c	202.45 ^b	215.71 ^a	196.73 ^b	2.18
ADFI (g)	1508.1 ^b	1574.55 ^a	1618.33 ^a	1580.4 ^a	19.63
FCR	0.117 ^d	0.129 ^b	0.133 ^a	0.124 ^c	0.001

¹BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; FCR, Feed Conversion Ratio.

²Numbers with different letters in each row differ significantly ($p < 0.05$).

³SEM: standard error of mean, where $n = 7$ per treatment

Conclusion The obtained results showed that lambs fed the control diet containing 0.0884 mg Co/kg DM had a reduced appetite and gained less weight than the supplemented animals, suggesting that the inclusion level of 0.0884 mg/kg DM Co was inadequate for normal growth of Mehraban lambs. Supplementation with 0.50 mg Co/kg DM seems to be optimum level for enhancing performance in lambs.

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Investigation on the effects of adding Direct-fed Microbial on total tract apparent nutrient digestibility in sheep

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Introduction Growing concern over the use of antibiotics and other growth stimulation in the animal feed industry has increased interest in evaluating the effects of Direct-fed Microbial (DFM) on animal performance (Sullivan and Martin, 1999). Some bacterial direct-fed microbials also seem able to function in the rumen. Depending on the species or combination of species, they can increase digestibility. Modes of action are variable and include native rumen microbiota enhancement through increased substrate breakdown, enhancement of nutrient uptake, stabilising rumen pH and provision of growth factors. The objectives of this study were to evaluate the effects of DFM supplementation on total tract digestibility.

Materials and methods An experiment was conducted as a crossover design with four periods and four diets. Four sheep were used in this experiment. The length of each period was 24 days, with 2 wk adaptation and 10 days measurement period. Sheep were kept in individual metabolic crates and fresh water was permanently available in the crates. Individual sheep BW was recorded before starting of each period. The experimental diet contained two basic diets which were similar except in sources of roughage (tables 1, and 2). DFM was granule shape and contained *Saccharomyces cerevisiae* (1×10^{11} cells), *Lactobacillus acidophilus* and *Lactobacillus casei* (5×10^9 CFU), *Enterococcus faecium* (5×10^9 CFU) and *Bacillus subtilis* (1×10^{10} CFU). DFM was added (5 g) to the control diets (TMR, 50% forage: 50% concentrate, dry matter basis) just before feeding. These diets met energy and protein requirements according to CNCPS standards and about two times of maintenance. Feed was offered twice daily at 0800 and 1600. Daily samples of feed were collected for chemical analysis for periods of 10 d throughout the experimental period. During the collection periods, the total weight of feces was recorded daily, and then a 10% sample was collected and composited by period and treatment. Samples of feces and feed were frozen at -20°C until analysis. Feed and feces were dried in an air-forced oven at 60°C for 48 h and then were ground through a 1-mm screen and analyzed for DM, OM, CP and fat according to AOAC (1995). The method of Van Soest *et al.* (1991) used to analyze ADF and NDF. Data for total tract apparent nutrient digestibility was analyzed as a crossover design with sheep, diets, periods and weight.

Table 1 Ingredients of experimental diets (%)

Item	Diet 1	Diet 2
Alfalfa hay	36.5	0
Corn silage	0	36.5
Wheat straw	15	15
Barley ground	45.7	37.6
Canola meal	2	9.5
Calcium carbonate	0.01	0.08
Mineral/vitamin mix	0.05	0.04
Salt	0.02	0.02

Table 2 Chemical composition of experimental diets

Item	Diet 1	Diet 2
ME (Kcal/d)	2.343	2.356
MP (g/d)	89	88
CP (%)	11.4	11.6
NDF (%)	41.8	42.2
Ash (%)	6.4	8.4
Ca (g/d)	6	5
P (g/d)	3	3

Results The results of apparent digestibility are presented in Table 3. Addition of DFM to diets did not affect apparent digestibility of DM, OM, NDF and ADF, however, digestibility of CP and EE in sheep that received diets containing corn silage were lower and higher than alfalfa hay, respectively ($P < 0.05$).

Table 3 Nutrient digestibility of diets without or with DFM (%)

Item	1	2	3	4	SE
DM	64.6	72.2	65.7	75.2	1.5
OM	62.71	69.26	65.32	72.26	1.2
CP	61.78 ^a	65.45 ^a	50.34 ^b	54.61 ^b	1.02
NDF	56.67	60.8	60.34	66.18	1.23
ADF	47.74	50.23	49.85	53.85	2.16
EE	54.4 ^b	55.9 ^b	63.3 ^a	64.2 ^a	1.7

1, control containing alfalfa hay as source of roughage; 2, diet 1 with DFM; 3, control containing corn silage as source of roughage; 4, diet 3 with DFM

Conclusion Results show that addition of this mixture of microorganisms did not affect nutrient digestibility, however, different sources of forage could affect on digestibility, as digestibility of CP and EE in sheep that received diets contain corn silage were lower and higher than alfalfa hay, respectively ($P < 0.05$).

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Chemical composition, organic matter degradation and metabolisable energy of three non-cultivated pastures located in the central plateau of Mexico

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Introduction Approximately 80% of all production costs are related to the purchase of forages and other feeds in dairy enterprises in Mexico. Resource poor, smallholder (*campesino*) systems are heavily reliant on the use of maize stover, non-cultivated/cultivated grass species and weeds to feed their dairy cattle, thus a nutritional understanding of local forage resources would be of considerable economic benefit. The objective of this study was to determine the nutritive value of non-cultivated pastures (NcP) during one productive year.

Materials and methods Three NcP designated as 1, 2 and 3 of which two were multi-species (1 and 2) and one dominated by kikuyu grass (*Penisetum clandestinum*) (3), located at the Toluca valley in the central highlands of Mexico (19° 27' N and 99° 38' W), were selected for study. Thirty samples were collected from each pasture per month, for 12 months from summer 2006 to end of Spring 2007 (i.e. total samples per pasture = 360). All standing plant material within a 0.50 m² quadrant was cut 5 cm above ground level and immediately dried at 60°C until constant weight. The sampling area was marked with pegs in order to ensure that samples were taken from the same area on each occasion. Plant samples were analysed for dry matter (DM), organic matter (OM) and crude protein (CP; nitrogen x 6.25; AOAC, 1990). Neutral and acid detergent fibre (NDF and ADF) were estimated according to the method of Van Soest *et al.* (1991). Metabolisable energy (ME) was estimated from organic matter degradation (OMd) (AFRC, 1995). Data were analysed using Minitab® (v. 15) as a complete block design, with season (S) as treatments and pastures (P) as blocks.

Results Significant differences (P<0.001) were observed for the effects of S and P as well as the interaction S x P (Table 1). Contents of DM and NDF were highest in the spring in pasture 1, and highest in the winter in pasture 2 and 3. For CP and ME, pasture 1 had the highest contents in summer, while the values were highest for pasture 2 and 3 in spring and summer. OMd varied across seasons; summer and winter had the highest and lowest values respectively and varied between pastures with pasture 3 having higher values than pasture 1 and 2. Pasture 1 had the lowest OMd values in all seasons.

Table 1 Chemical composition and ME of non-cultivated pastures

Pasture type	Pasture No.	Season	DM (g/kg)	OMd (g/kg DM)	CP	NDF	ME (MJ/kg DM)
Multi-species	1	Su	320	489	109	743	7.1
		Au	276	397	84	790	5.7
		Wi	430	412	87	790	6
		Sp	570	399	90	789	5.7
Multi-species	2	Su	315	559	90	697	8.3
		Au	440	487	74	767	7.2
		Wi	740	461	63	793	6.8
		Sp	435	524	103	756	7.7
Mono-species	3	Su	229	591	159	749	8.9
		Au	389	556	159	740	8.4
		Wi	724	481	113	760	7
		Sp	280	556	216	746	8.4
		s.e.m.	49	19	13	8	0.3
		S	***	***	***	***	***
		P	***	***	***	***	***
		S x P	***	***	***	***	***

*** (P<0.001), Su summer, Au autumn, Wi winter, Sp spring, s.e.m. standard error of the mean

Conclusions Chemical composition and ME concentration is influenced by time of the year (i.e. season) and pasture type. In this study the forage material from pasture 3 had a higher nutritional value than pastures 2 or 3, which may be caused by differences in soil type and fertility as well as species composition. Further work is needed to characterise forage sources used by smallholder farmers in Mexico.

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Behavior analysis of a first order degradation model when fitting the nutrient ruminal disappearance data

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Introduction There are several different models for describing the nutrient ruminal degradation of feeds. The use of a particular model in fitting a given degradability data set and estimating the degradability parameters implies the holistic examination of model goodness-of-fit, otherwise the validity of estimated parameters may be controvertible. One of the criteria for evaluating goodness-of-fit is model behaviour analysis. In the present work, the behaviour of a first-order model when fitting whole soybean ruminal degradability data was investigated.

Materials and methods DM and CP degradability data of two Iranian soybean cultivars (Sahar and Williams) as raw, roasted and steep-roasted (6 feeds in total), which was recorded at fixed incubation periods (1, 2, 3, 4, 8, 16, 24, 36 and 48 h) for each feed and yielded a total of 6 disappearance curves, were used in this study. The evaluated model was a rational function or inverse polynomial (linear over linear), which describe a rectangular hyperbola and assumes a variable fractional rate of degradation that declines with time (Lopez *et al.*, 1999). Fractional degradation rate (/h), disappearance to

time t (%) and extent of degradation (%) were calculated using the formulae $\frac{1}{t-L+T}$, $a+b\frac{t-L}{t-L+T}$ and

$a+bk\int_L^{\infty}\left(\frac{t-L}{t-L+T}\right)e^{-kt}dt$, respectively. L is a discrete lag parameter (h) and was included in the model to represent the

time interval before degradation commences, T is a time constant (h), and a , b and k are rapidly soluble fraction (%), slowly degradable fraction (%), and fractional passage rate (/h), respectively. The model was fitted to the DM and CP disappearance curves by nonlinear regression using the PROC NLIN of SAS to estimate ruminal degradation parameters. Model behaviour analysis was used to evaluate general goodness-of-fit (quality of prediction) of the model to each curve.

Results All curves could be fitted by the model using nonlinear regression and utilizing the PROC NLIN of SAS, as convergence to a solution occurred in all cases and the degradability parameters could be estimated (Table 1). However, when the model was unconstrained, $(a+b)$ tended to a value larger than 1 which was unacceptable biologically, so the sum of these two parameter was constrained to be no greater than unity. Although non-convergence was not found, a large number of iterations were required and significant sensitivity to starting values was observed, which were symptomatic of an ill-conditioned or inappropriate model.

Table 1 Parameter estimates (SE in parentheses) of DM and CP ruminal disappearance of all feeds¹

Item ²	DM	CP
a	47.67 (1.672)	45.17 (4.088)
b	52.16 (1.625)	55.25 (3.014)
U	0.17 (0.410)	-0.42 (1.578)
T	11.80 (3.784)	13.83 (5.562)
L	2.65 (2.850)	2.81 (2.833)
E ($k = 0.06$)	64.23 (8.786)	58.46 (9.937)
E ($k = 0.08$)	60.37 (8.326)	55.09 (9.556)

¹rather than present the parameter estimates for each rumen incubated feed, the mean of the parameters estimated over all incubated feeds are shown.

² a , rapidly soluble fraction (%); b , slowly degradable fraction (%); U , undegradable fraction (%), calculated as $(1 - a - b)$; T , time constant (h), such $L + T$ is the time taken for half of the slowly degradable fraction to disappear; L , lag time (h); E , extent of degradation (%); k , fractional passage rate (/h).

Conclusion Although the model could estimate the parameters of the DM and CP degradability data for the feeds used in this study and the values presented in Table 1 apparently are acceptable, the behaviour of the model when fitting reflected its inadequacy for this particular data set. It can be concluded that before presenting estimated parameters of models, examination of model goodness-of-fit through model behaviour analysis is necessary.

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Evaluation of mathematical models to describe protein fraction degradation kinetics of various oilseed meals

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Introduction *In situ* procedure is a direct method of measuring the rumen degradation kinetic of a feed nutrient. Data obtained by this technique are generally analysed using an exponential curve (Ørskov and McDonald, 1979). However, very low attention has been paid to choice of mathematical model to fit the curves and goodness-of-fit of the model. Lopez *et al.* (1999) pointed out that the disappearance of some feed components, particularly structural carbohydrates, exhibits a larger variety of forms than for crude protein (CP). In the present study, two different mathematical models of a straight line or a negative exponential (France *et al.*, 1990; and Ørskov and McDonald, 1979) were selected to evaluate *in situ* degradation kinetics of protein fractions including true protein (TP), neutral-detergent insoluble protein (NDIP) and acid-detergent insoluble protein (ADIP) of various oilseed meals (cottonseed meal (CSM), soyabean meal (SM) and rapeseed meal (RM)).

Materials and methods Samples of various oilseed meals including CSM, SM and RM were collected from oil industries located in east Iran during May-September, 2006. Four Holstein steers (330±15 kg body weight) were used. Animals fed a diet consisted of 1.7 kg wheat straw, 3.8 kg lucerne hay and 3.4 kg of a commercial concentrate (CP: 184 g/kg dry matter (DM)) at 0800 and 1800 h. In order to determine protein fraction degradability coefficients, 5 g DM equivalent of each sample (ground with a 2-mm screen mill) was placed in individual polyester bags (50 µm pore size and averaged 12 cm × 19 cm). Incubation times were 2, 4, 8, 16, 24, 48, 72 and 96 h. Bags were placed in the dorsal sac of the reticulorumen of each steer (2 bags per each sample in each steer). Immediately after incubation, the bags were hand washed thoroughly in cold running water. Two bags of each feed sample were washed without incubation in the rumen (0.0 h samples). The bags were dried in a forced-air oven (58 °C, 48 h). Crude protein and protein fraction concentrations of the rumen pre-incubated and incubated samples were determined as proposed by Licitra *et al.* (1996). Data of protein fraction degradations were adjusted to a straight line model [model I, $P=a+ct$, France *et al.*, 1990] or a negative exponential model [model II, $P=a+b(1-e^{-ct})$, Ørskov and McDonald, 1979], where P = fraction degraded in the time t , a = rapidly degradable fraction, b = slowly degradable fraction, c = fractional degradation rate and t = incubation time. Lag phase was not included in the models. Several statistics, including mean square prediction error (MSPE), root of MSPE (rMSPE, expressed as a percentage of the observed mean) and coefficient of determination (R-square) were used to evaluate goodness-of-fit of each model.

Results The results of protein fraction degradation kinetics of each sample using models I and II are shown in Table 1. In addition, for each model, the MSPE, rMSPE and R-square have been presented.

Table 1 *In situ* protein fraction degradation parameters estimated for various oilseed meals using models I ($P=a+ct$) and II ($P=a+b(1-e^{-ct})$)

Oilseed meals	Model I							Model II					
	Protein fractions	a	b	c	MSPE	rMSPE	R ²	a	b	c	MSPE	rMSPE	R ²
CSM	TP	0.61	0.33	0.06	0.0017	4.1	0.91	0.58	0.42	0.10	0.0011	3.1	0.96
	NDIP	0.18	0.67	0.01	0.0021	4.5	0.88	0.25	0.57	0.01	0.0012	3.4	0.94
	ADIP	0.36	0.40	0.09	0.0030	5.4	0.91	0.35	0.34	0.12	0.0009	3.0	0.97
SM	TP	0.36	0.66	0.06	0.0019	4.3	0.89	0.40	0.87	0.10	0.0015	3.8	0.96
	NDIP	0.06	0.71	0.04	0.0015	3.8	0.90	0.03	0.82	0.04	0.0014	3.7	0.98
	ADIP	0.01	0.83	0.06	0.0021	4.5	0.89	0.05	0.91	0.06	0.0016	4.0	0.94
RM	TP	0.36	0.44	0.06	0.0015	3.8	0.91	0.43	0.51	0.07	0.0014	3.7	0.98
	NDIP	0.11	0.61	0.04	0.0021	4.5	0.90	0.16	0.71	0.05	0.0018	4.2	0.99
	ADIP	0.10	0.33	0.05	0.0015	3.8	0.90	0.15	0.29	0.08	0.0012	3.4	0.97

Conclusions There were major differences among the oilseed meals for the different protein fraction degradation kinetics evaluated in the present study. The low values of ADIP degradation confirmed the previous results. In the CNCPS model, ADIP is believed to be more slowly degraded in the rumen than are TP and NDIP (Sniffen *et al.*, 1992). Results of the present experiment showed that, based on various statistical tests, MSPE and rMSPE, as indicators of model accuracy, of model II gave better fits to protein fraction degradation kinetics than model I. R-square showed that the variation was high for models I compared with model II, and values of rMSPE of Model II was sufficiently small to show that model parameters accurately. Therefore, the negative exponential model is well suited to describe the degradability patterns of protein fractions of TP, NDIP and ADIP.

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Ruminal crude protein degradability parameters of some feedstuffs using *in situ* technique

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Introduction The *in situ* technique has been used widely for estimating ruminal nutrient degradation and to provide estimates of both the rate and extent of disappearance of feed constituents (Mehrez and Ørskov, 1977), because it is a relatively simple, low-cost method compared with methods involving intestinally cannulated animals. This technique provides a useful means to estimate rates of disappearance and potential degradability of feedstuffs and feed constituents. The technique involves suspending bags containing different feedstuffs in the rumen and measuring nutrient disappearance at various time intervals. Hence, it also may provide an advantage compared with laboratory methods because it involves digestive processes that occur in the rumen of a living animal; however, several factors affect estimates of nutrient digestion and need to be controlled for this technique to be standardised. 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 estimate *in situ* crude protein degradability of dried grape by-product (DGB), noodle waste (NW), tomato pomace (TP), apple pomace (AP) and grape pomace (GP) using the *in situ* technique.

Materials and methods *In situ* degradation characteristics were determined (Ørskov and McDonald, 1970) in 4 replication in two rumen fistulated sheep (38±1.5 kg). The sheep were fed twice daily (08 and 14 h) a diet containing 600 g/kg concentrate and 400 g/kg forage. Nylon bags (which were approximately 6×12 cm, 45µm) 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. After incubation times the nylon bags removed and washed in tap water until the water remained clear. There were four replications per treatment. The CP was measured according to AOAC (2005). The rate and extent of CP 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 were analysed using the ANOVA procedure of SAS (SAS Institute, 2002). The chemical composition of feeds was determined using the methods recommended by AOAC (2005).

Result The chemical compositions of feedstuffs are presented in Table 2. The results are shown in Table 1. There was a significant differences between treatment in incubation times ($p < 0.05$). The CP soluble fraction (a) for noodle waste was higher than the other feedstuffs (69.33%) and among the feedstuffs grape pomace and tomato pomace had the lowest fraction a. The fraction b for apple pomace was higher than the other feedstuffs (64.43%). The fractional rate of degradation (c) was 0.0316, 0.0306, 0.099, 0.0529 and 0.2934, respectively. Noodle waste had the highest effective degradability (ED) while grape pomace had the lowest. The results showed that the soluble fraction (a) and fraction c in NW were higher than the other feedstuffs (69.33% and 0.2934, respectively). CP disappearance of noodle waste (NW) at all incubation times was higher than other feedstuffs. Grape pomace had the lowest crude protein disappearance value within the feedstuffs at all incubation times.

Table 1 *In situ* disappearance(%) of CP*

Feeds	Time of incubation (h)										a	b	c (%/h)	ED
	0	2	4	6	8	12	16	24	36	48				
DGB	31.8 ^c	33.1 ^c	38.8 ^c	41.2 ^c	56.5 ^b	58 ^b	60.5 ^b	66.4 ^c	76.8 ^c	82.1 ^c	30.02	55.17	0.0529	70.1
GP	17.1 ^d	20.7 ^d	24.9 ^e	26.4 ^e	28.9 ^e	29.3 ^c	37.4 ^d	53.6 ^e	55.8 ^e	59.6 ^e	16.57	57.31	0.0316	51.7
NW	67.7 ^a	85.3 ^a	86.4 ^a	89.4 ^a	91.8 ^a	93.2 ^a	94.2 ^a	95.5 ^a	97.2 ^a	98.2 ^a	69.33	26.27	0.2934	93.9
AP	38.5 ^b	41.5 ^b	45.8 ^b	46.7 ^b	46.8 ^c	47.1 ^c	51.8 ^c	78.2 ^b	81.5 ^b	86.8 ^b	35.57	64.43	0.0306	74.5
TP	16.7 ^d	22.5 ^d	31.2 ^d	36.2 ^d	42.5 ^d	55.4 ^b	58.8 ^b	60.6 ^d	64.5 ^d	68.2 ^d	14.61	52.98	0.099	58.7
SEM	1.04	1.35	1.90	1.35	1.35	0.88	0.96	1.19	1.12	1.05	-	-	-	-

*The means within a column without common letter differ ($p < 0.05$).

Table 2 The chemical composition of feeds (%)

Items	Feeds				
	DGB	GP	NW	AP	TP
DM	88.5	93.3	90.8	92.3	96.5
CP	6.4	6.62	11.3	5.3	21.6
ADF	25.5	18.4	-	28	58.7

Conclusion In the present study, feeds composition significantly affected the degradation parameters, when ruminal CP degradation of the various feed samples were considered. Results of the present experiment indicated that the rapidly degradable fraction (a) of CP of GP and TP were lower than the other feeds.

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The effect of fluctuations in rumen pH on protozoa populations in rumen fluid as determined by real-time polymerase chain reaction

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Introduction Ruminal ciliated protozoa are similar to bacteria in that their numbers increase in response to increased substrate availability. Moderate increases in grain intake result in large increases in the numbers of ciliated protozoa (Dennis *et al.*, 1983). In contrast, high- or all grain diets have variable effects, including total elimination of protozoa in some animals (Franzolin and Dehority, 1996). Ciliated protozoa are believed to be much more sensitive than bacteria to fluctuations and reductions in ruminal pH; hence, ruminal pH is a critical factor in the maintenance of ciliated protozoa in the rumen. The objective of the present experiment was to investigate the effect of fluctuation in ruminal pH on the population of protozoa in rumen fluid.

Materials and methods Four Holstein steers (300 ± 15 kg, body weight) with rumen fistulae were fed experimental diets (7 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 0800 and 2000h. Animals had access to drinking water at all times. Ruminal fluid was taken, by suction, via 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 feeding (0.0h) until 8h post feeding at 15 min intervals. The samples of rumen fluid taken before the morning feed, and 4h post feeding were stored in liquid N₂ until used for protozoa 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. Protozoa rDNA concentrations were measured by real time PCR relative to total bacteria amplification ($\Delta\Delta Ct$). The 18s rRNA gene-targeted primer sets used in the present study were forward: GCTTTCGWTGGTAGTGTATT and reverse: CTTGCCCTCYAATCGTWCT. Cycling conditions were 95°C for 10 min, forty five cycles of 94°C for 10s, 55°C for 20s and 72°C for 15s; fluorescence readings were taken after each extension step, and a final melting analysis was obtained by heating at 0.1°C/s increment from 45 to 94°C, with fluorescence collection at 0.1°C at intervals. Data are expressed relative to quantification of the total bacterial population. Data were analyzed using the GLM procedure of SAS

($y = \text{Mean} + \text{Treatment} + \text{Animal} + \text{Period} + \text{Time} + \text{Time} \times \text{Treatment} + \text{Animal} \times \text{Time} + \text{residual}$) and the means compared by the Tukey test ($P < 0.05$).

Results Ruminal pH decreased, from before the morning feeding 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, ($P < 0.05$). rDNA concentration of protozoa in rumen fluid is shown in Figure 1. The population of protozoa in the ruminal fluid increased, when level of concentrate was increased ($P < 0.05$).

Figure 1 Protozoa (± SD) in rumen fluid before and 4h 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 protozoa in the free rumen fluid. These results are in contradiction with some studies that suggest that reduction in ruminal pH is a method for experimental defaunation in ruminants (Franzolin and Dehority, 1996). The reason for this contradiction is the protozoa numbers in the liquid phase are not determined solely by rumen pH, several factors seem to influence the concentration and composition of the protozoal fauna in the rumen. These include composition of diet, pH, turnover rate, frequency of feeding, and feed level (Franzolin and Dehority, 1996).

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***In vitro* rumen fungi growth in a medium containing sodium hydroxide treated wheat straw using competitive PCR assay**

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Introduction Rumen fungi produce a wide range of polysaccharide degrading enzymes during growth on cell walls and can degrade 0.25 - 0.65 of plant tissue dry weight in pure cultures (Orpin, 1983). It has been proposed that sodium hydroxide may break-down hemicellulose and cellulose and expose them to microbial attachment and improve digestibility (Goto *et al.*, 1993). The aim of the present study was to determine *in vitro* rumen fungal growth using a quantitative competitive PCR assay (QC-PCR) and dry matter (DM) and neutral detergent fibre (NDF) disappearance from a medium containing untreated (US) or sodium hydroxide treated wheat straw (TS).

Materials and methods Wheat straw was incubated in the rumen of ruminal fistulated sheep, fed 250 g concentrate and 750 g lucerne hay, to provide rumen fungi isolates. Fungi were grown under anaerobic conditions at 39 °C for 3 days (Joblin 1981). These isolates were used (1:9, isolates: medium) as a fungal inoculum. Serum bottles containing fungi culture medium, 1 g straw, untreated or treated with 10 g NaOH/ kg DM, and 1 ml antibiotic solution, were used to culture the isolated fungi at 39 °C. After sub-culturing three times, isolated fungi were incubated for 1, 3, 9 and 12 days (3 replicates per time). After each incubation time, pH was determined and residual substrate filtered and analysed for DM and NDF. Relative quantitative detection of rumen anaerobic fungi population was conducted by QC-PCR according to Nassiry *et al.* (2008). Data for DM and NDF disappearance and pH were analysed as repeated measurements using the General Linear Model procedure of SAS (2001). The result of QC-PCR assay was quantified by ImageJ 1.29x and expressed in arbitrary units. Statistical analyses of the changes of fungal growth were by analysis of ANOVA and ANCOVA using the GLM procedure of SAS (2001).

Results Fungal growth and disappearance of DM and NDF of TS (Figure 1) was significantly higher than US. The lowest pH was detected in medium containing TS ($P < 0.01$).

Figure 1. *In vitro* pH and DM and NDF disappearances of a medium containing untreated (US) or sodium hydroxide treated wheat straw (TS: 10 g NaOH/kg DM).

Conclusions Results confirmed the data of Rezaeian *et al.* (2004) when NaOH-treated WS was incubated with rumen fungi dry matter losses were 38%. In addition, it was concluded that sodium hydroxide might increase fungal biomass and improve DM and NDF digestion of WS under *in vitro* conditions

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***In vitro* rumen fungi quantification in medium containing sodium hydroxide or formaldehyde treated sunflower meal using quantitative competitive PCR assay**

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Introduction The rumen anaerobic fungi are primary colonizers of fibrous plant materials that degrade lignin-containing plant cell walls. With the advancement of molecular enumeration methods, in particular 18S rDNA gene probing methods, researchers were able to monitor fungal species within the rumen (Stahl *et al.*, 1998). Quantitative competitive PCR (QC-PCR) techniques play an important role in nucleic acid quantification because of their significant lower cost of equipment and consumables (Franz *et al.*, 2001). The aim of this study was to use the QC-PCR assay to evaluate the relative quantitative comparison of anaerobic fungi population in medium containing sunflower meal, either untreated or treated with formaldehyde (3 and 6 g/kg DM) or sodium hydroxide (40 g/kg DM).

Materials and methods Rumen fungi were isolated from pre-incubated wheat straw in the rumen of rumenal fistulated sheep and then method of Joblin (1981) was used to grown under anaerobic conditions at 39 °C for 3 days (Joblin, 1981). These isolates were used (1:9) as a source of fungi inoculum. Serum bottles containing fungi culture medium, 1 g of sunflower meal as untreated (USM) or treated with 3 (F1SM) and 6 g (F2SM) formaldehyde/kg DM or 40 g NaOH/kg DM (SHSM) and 1 ml antibiotic solution were used to culture the isolated fungi at 39 °C in an incubator. To preparing fungi pure culture, subculturing was done three times and the fungi were identified using rumen anaerobic fungi primer (GAF) in pure culture. Total genomic DNA was isolated from pure culture samples using Guanidine Thiocyanate-Silica Gel method. A universal PCR primer pair GAF (F): 5'-GAG GAA GTA AAA GTC GTT AAC AAG GTT TG-3' and GAF(R): 5'-GAA ATT CAC AAA GGG TAG GAT GAT TT-3' was used to amplify a specific region of 18S rDNA from anaerobic rumen fungi. Standard control DNA was constructed to use in the QC-PCR and was shown to amplify under the same reaction condition and the same amplification efficiency as the target DNA. The PCR was performed in a final volume of 25 µl sealed in a capillary tip, and thermocycling was carried out in a model 2000 (Biometra). The PCR amplification condition was as follows: denaturation at 94 °C for 4 min followed by 35 cycles of 94 °C for 30 s; 56 °C for 30 s; and 72 °C for 1 min followed by a final extension at 72 °C for 5 min. The PCR products were separated by electrophoresis on agarose gels, stained with ethidium bromide, and visualized by UV transillumination. The relative intensities of PCR products were used to compare fungal biomass under different samples. The signal intensity was quantified by ImageJ 1.29x and expressed in arbitrary units. The data was analyzed using the GLM procedure of SAS (2001) for a completely randomized design.

Results The competitive PCR reaction for DNA extracted from fungi media is given in Figure 1. The result of QC-PCR (Figure 2) showed that the growth of rumen fungi in the medium containing sunflower meal treated with NaOH was greater than the other treatments ($P < 0.05$). Formaldehyde decreased the growth of rumen fungi.

Figure 1 Competitive PCR reaction for DNA extracted from fungi medium

Figure 2 Alteration in rumen fungi population in medium containing: untreated sunflower meal (USM); 40 g NaOH/kg DM treated sunflower meal (SHSM); 3 g formaldehyde/kg DM treated sunflower meal (F1SM); 6 g formaldehyde/kg DM treated sunflower meal (F2SM), log ratio of intensities of amplified target DNA to standard (Log (T/C))

Conclusions The method of QC-PCR for enumerating anaerobic rumen fungi in the present study demonstrated that the treatment of sunflower meal with NaOH increased fungal growth, while formaldehyde decreased it. Increasing rumen fungi populations and avicelase enzyme and following digestibility with NaOH was reported by Chen *et al.* (2007). Sodium hydroxide might hydrolyze the ester bonds between lignin and hemicellulose and expose the cellulose to microbial attachment and improve digestibility (Goto *et al.*, 1993). The data obtained in the present study indicated the ability of QC-PCR to determine the quantity of rumen fungi using *in vitro* culture.

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Evaluation of a zero-order model to describe ruminal degradation kinetics of whole soybean incubated *in situ*

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Introduction *In situ* incubation of feeds in the rumen serves as a basic procedure in many feed evaluation systems. The formulation of hypotheses concerning underlying biological concepts of ruminal degradability has led to development of different mathematical models describing the resultant time course disappearance curves of feed fractions. The use of models allows comparison of parameter estimates (or combinations thereof), which ideally reflect these biological concepts, between feeds or feeding systems. Among different factors affecting the experimental measurements of *in situ* ruminal disappearance of feeds, less attention has been paid to choice of mathematical model to fit the curves and the goodness-of-fit of the model. The objective of the present work, therefore, was to evaluate the ability of a zero-order mathematical model to describe *in situ* disappearance curves obtained with whole soybean, and to give adequate estimates of the parameters needed to determine extent of ruminal degradation.

Materials and methods Two Iranian cultivars of soybeans (Sahar and Williams) as raw, roasted and steep-roasted were used in this study. Degradability of DM and CP was recorded at each incubation period for each of 6 feeds, yielding a total of 12 disappearance curves, obtained from the following time periods of incubation in rumen: 0, 2, 4, 8, 16, 24, 36, 48 and 72h. The evaluated model was a segmented model with three spline-lines delimited by two nodes or break points, constraining splines 1 and 3 to be horizontal asymptotes, and follows zero-order degradation kinetics (France *et al.*, 1990). Fractional degradation rate (/h), disappearance to time t (%) and extent of degradation (%) were calculated using the formulae $\frac{c}{b-c(t-L)}$, $a + c(t-L)$ and $a + \frac{c}{k} \ln \frac{kb + ce^{kl}}{c} - cL$, respectively. L is a discrete lag parameter and was included in

the model to represent the time interval before degradation commences and a , b , c and k are rapidly soluble fraction (%), slowly degradable fraction (%), the constant degradation rate (%/h) and fractional passage rate (/h), respectively. The model was fitted to the DM and CP disappearance curves by nonlinear regression using the PROC NLIN of SAS to estimate ruminal degradation parameters. A number of statistics, including mean square prediction error (MSPE), root of MSPE (rMSPE) expressed as a percentage of the observed mean and coefficient of determination (R-square) were used to evaluate general goodness-of-fit (quality of prediction) of model to each curve.

Results The results for parameters estimated, MSPE and R-square calculated for the model are shown in Table 1. The value of rMSPE was not significantly different between non-lagged and lagged version of model for both DM and CP components and was sufficiently small to show both forms were able to estimate model parameters accurately. Moreover, decomposition of MSPE gave similar values of ECT (error in central tendency), ER (error due to regression) and ED (error due to disturbance) for both versions, and was mainly dominated by the disturbance component, which indicates that ruminal degradation of DM and CP of samples was well represented by both lagged and non-lagged versions.

Table 1 Parameter estimates for lagged and non-lagged versions of model (SE in parentheses) and statistics calculated for comparison of the two equations

Item	Lagged version		Non-lagged version	
	DM	CP	DM	CP
Parameter estimates ¹				
a	50.27 (2.523)	43.64 (4.686)	47.96 (1.464)	43.07 (3.737)
b	45.95 (2.753)	50.72 (1.828)	47.72 (2.645)	51.35 (2.525)
c	0.012 (0.0027)	0.013 (0.0030)	0.015 (0.0078)	0.016 (0.092)
U	3.79 (2.241)	5.64 (3.606)	4.32 (1.423)	5.58 (3.084)
L	1.30 (2.195)	0.0 (8.2 × 10 ⁻⁷)	...	...
E ($k = 0.06$)	73.40 (4.206)	69.31 (6.584)	74.06 (4.820)	70.68 (8.658)
E ($k = 0.08$)	70.50 (4.223)	66.22 (6.624)	71.18 (4.734)	67.62 (8.719)
R-square (%)	98.79 (2.250)	97.06 (3.82)	97.40 (0.782)	96.32 (2.613)
rMSPE	4.17 (1.341)	5.36 (1.795)	4.36 (0.666)	5.66 (1.894)
MSPE analysis (%MSPE)				
ECT	0.16 (0.209)	5.74 (6.411)	0.01 (0.012)	4.31 (7.647)
ER	1.49 (2.202)	1.11 (1.755)	0.03 (0.009)	4.69 (8.048)
ED	98.35 (2.263)	93.15 (7.140)	99.96 (0.020)	91.00 (15.687)

¹ a , rapidly soluble fraction (%); b , slowly degradable fraction (%); U , undegradable fraction (%), calculated as $(1 - a - b)$; L , lag time (h); E , extent of degradation (%); k , fractional passage rate (/h).

Conclusion The results showed that the three-piece linear model was suited to describing the degradability patterns of whole raw and roasted soybeans.

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The effect of non-structural carbohydrate on *in vitro* first order disappearance kinetic of cellulose

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Introduction Ruminal cellulose digestion is a complex microbial process that involves adhesion of microbial cells to cellulose, cellulose hydrolysis, and fermentation of the resulting celldextrins to volatile fatty acid, methane, and CO₂ (Weimer, 1996). Information about how cellulose digestion and fermentation are affected by different ruminal environmental conditions is necessary to understand ruminant performance (Mourino, 2001). The aim of the present study was to elucidate the impact of type of supplemental non-structural carbohydrates (NSC) (starch and/ or sucrose) on *in vitro* first order disappearance kinetic of commercial cellulose (Cell).

Materials and methods Experimental treatments consisted of non-supplemented Cell (150 mg) and Cell plus NSC (70 mg) as sucrose (Su) or starch (St) or a 1:1 mixture of Su and St (Su+ St). Treatments were incubated in a medium prepared as described by Arroquy *et al.* (2005). Forty five ml of medium were distributed into 100 ml bottles. Then, each bottle was inoculated with 5 ml of strained rumen fluid and finely bubbled with CO₂. The bottles (three bottles per each treatment per each time) were incubated for 24, 48, and 96 h at 39 °C. For each time, three bottles containing just medium was run as blanks. Then, each bottle contents were filtered through a 22 µm filter, and the amount of the unfiltered Cell was determined. Data were analyzed as a complete randomized design using a 4 × 3 factorial arrangement. The first factor consisted of supplemental NSC type and the second factor consisted of incubation time. Data of the disappearance rate of Cell were analysed using general linear model procedure of SAS (2003). Non-linear first order model was used to estimate the digestion kinetic parameters of Cell. The model was: $D_{(t)} = D_{(i)} \cdot \exp(-k \cdot \text{time}) + I$; Where, $D_{(i)}$ is potentially digestible fraction of Cell, $D_{(t)}$ is potentially digestible residues, k is fractional rate constant of digestion (h⁻¹) and, I is indigestible fraction.

Results Inclusion of NSC in the fermentation mixture resulted in a significantly larger depression in the extent of Cell disappearance with regard to the control (no NSC supplemented Cell), and this effect was more obvious for St supplementation (Fig. 1). However, the NSC effect on extent of Cell disappearance was most clearly evident at the time periods from 0 to 48 h of incubation. The fractional rate of Cell disappearance (Fig. 2) was greater ($P < 0.05$) in control treatment compared with those treatments containing NSC. However, the degree of depression in Cell fractional rate was dependent on the type of supplemental NSC used. When St or Su+ St was served as the source of supplemental NSC, the rate of Cell disappearance was significantly decreased. In contrast, the fractional rate of Cell disappearance for the Su treatment was not significantly influenced. Indigestible fraction of Cell was greater ($P < 0.05$) for treatments receiving Su or Su+ St as the source of supplemental NSC, when compared with the control.

Figure 1 Effect of supplemental NSC on *in vitro* cellulose disappearance.

Figure 2 Effect of supplemental NSC on *in vitro* fractional rate of Cellulose digestion (K_d , h⁻¹) and indigestible fraction (I , g⁻¹). Cell= Cellulose, Cell+ Su= Cellulose+ Sucrose, Cell+ St= Cellulose+ Starch, Cell+ Su+ St= Cellulose+ Sucrose+ Starch

Conclusions *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. Under the *in vitro* condition that was employed in the present study, the types of supplemental NSC examined have different effect with regard to their effect on Cell disappearance, and starch had more negative effect than sucrose. However, it is a need to evaluate the effect of NSC on ruminal cellulose disappearance under *in vivo* condition.

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What has happened to the genetic merit of average UK Holstein cows over the last 24 years?

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Introduction Genetic trends can be readily calculated from national genetic evaluation data. However, these reflect the combined decisions of all owners of recorded dairy cattle rather than a single policy followed on one farm over a long period of time. The Langhill herd maintained by SAC at its Crichton Royal Farm allows the study of the genetics of cows of average merit over a twenty-four year period.

Materials and methods Cows in this study were the Control Line cows from the Langhill herd. Their management has been previously described by Pollott and Coffey (2008). These cows were the daughters of sires chosen to be of average genetic merit at the time of AI. In this case average genetic merit was defined as zero PIN. The PTAs of these cows were obtained from the national genetic evaluations carried out in April 2005. These PTAs were summarised by year of birth and analysed to investigate the trends in a range of production and fertility traits. Both linear and single-spline regression models were used to analyse the data.

Results The genetic trend in average £PIN value is shown in Figure 1; genetic merit remained constant from 1980 to 1994 when it started to rise at a rate of 4.44 £/year ($SE \pm 0.614$). A similar pattern of change was noted in the mean PTA of a number of other traits, namely 305d milk yield ($46\text{kg} \pm 7.56$), test-day yield at day 110 of lactation ($0.303\text{kg} \pm 0.0225$) and fat plus protein yield ($3.01\text{kg} \pm 0.412$). In contrast the fertility traits showed a linear decline throughout the whole period. Calving interval increased by $0.658\text{d}/\text{year}$ (± 0.0336), non-return rate at 56d decreased by $-0.00206/\text{year}$ (± 0.000224), the number of services increased by $0.00575/\text{year}$ (± 0.000493) and day of first service increased by $0.330\text{d}/\text{year}$ (± 0.0231). Body condition score showed a linear decrease of $-0.0544\text{units}/\text{year}$ (± 0.00328) and stature increased by $0.0621\text{units}/\text{year}$ (± 0.00923); both traits measured on a 9 point scale.

Figure 1 The genetic trend in PIN for Control Line cows (1980-2004).

Conclusions Cows of average PIN value exhibited a stable genetic merit trend until the base of evaluations was set to change in 1995; thereafter genetic merit increased as the base was changed. This pattern was reflected in all analysed milk traits as well. By contrast, the decline in fertility noted by several authors at national and regional level (Butler and Smith, 1989; Royal *et al.*, 2000; A.-Ranberg *et al.*, 2003) was demonstrated throughout the whole period covered by these analyses and was not specifically linked to increasing performance. However, this decline in fertility was always less than that reported for the Selection Line cows in this herd (Pollott and Coffey, 2009). The average cow has become leaner and taller between 1980 and 2004 and this may be a reflection of the increase in Holstein genes in this herd. Control Line cows had an average of 25% Holstein genes in 1980 and this had increased to 98% by 2004.

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A proposed novel breeding system for intensively reared dairy flocks.

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Introduction Dairy sheep production is an important industry in Greece and other Mediterranean countries. The traditional system of lambing once a year, as well as the size of the flocks and the extended lactation period has been an obstacle to implement accelerated breeding systems that could capitalise on the full potential of the ewe (Fahmy and Lavallee, 1990). The objective of this study was to develop a management system which combines an accelerated breeding scheme and grouping of ewes in relation to their milk yield, for large, intensively reared, dairy flocks.

Materials and methods A large commercial flock located at Magnesia, Central Greece was used. It comprised of 630 ewes of the Chios breed that were reared as five lambing groups with lambing all year round (every 2 months). At the commencement of the experiment (February 2008) the ewes were reallocated into five new groups based on their milk yield. Individual milk yields were recorded daily and data for each ewe were assessed every 2 months according to the level of milk yield for the previous 3 test days. The ewes were kept in designated areas of the barn allowing their flow into different groups. All lambs produced were reared artificially. The proposed breeding system is depicted in Figure 1. The underlying idea is that there is a rotation of ewes from one group to other every 2 months depending on their milk yield. The groups were: (i) **High:** Ewes in the first 2 month of lactation and those yielding >1.5lt of milk, (ii) **Medium:** Ewes yielding 1-1.5 lt of milk (iii), **Low 1:** comprised of ewes yielding <1 lt of milk. Hormonal oestrous synchronisation with intravaginal sponges was performed as soon as the ewes entered the Low 1 group. Breeding commenced 14 days later. Thereafter, teasers were used to indentify ewes that failed to conceive. They remained in Low 1 and were subjected to hormonal synchronisation of oestrus until they conceived. When pregnancy was confirmed with ultrasound the ewes remained in Low 1 for two months before moved to the next group - (iv) **Low 2:** comprised of ewes being in the last 45 days of milking. In this group, a second ultrasound scanning was performed to confirm pregnancy and number of embryos, (v) **Dry period group:** all ewes two months before parturition.

Figure 1 Flow diagram of the proposed breeding system.

Results The implementation of the above system in the experimental flock resulted in a significant reduction of feed intake whereas the milk yield of ewes increased about 150 ml/ewe/day, which is 10% of the total daily milk yield of the average ewe. Although the latter are preliminary results the advantages and disadvantages of the proposed breeding system are summarised in Table 1.

Table 1 Advantages and disadvantages of the proposed breeding system

Advantages	Disadvantages
Continuous supply of lambs	Requirement of personnel with high technical skills
Milk with standard quality is produced throughout the year	Appropriate only for large intensively reared flocks
Efficient feeding management	Stress for the ewes that change group every 2 months
High yielders can be identified for genetic improvement.	Fewer births from ewes that persist on milk production
More than one lambing per year for low production ewes	Possible difficulties on fertilization of ewes during anoestrous could compromise the system
Preventive measures (eg. vaccines) are done on group basis	
Ewes that fail to conceive, rebred only 2 or 4 months later	

Conclusion Increasing productivity and lowering costs is a major concern for the dairy sheep farmers. Hence, accelerated breeding systems that increase milk production need to be developed. The proposed breeding system aims to improve the productivity of intensively reared dairy flocks. However, further work is needed firstly, to estimate whether the increase in productivity and the lower of the feeding cost, overcomes the increase in labour, facilities and equipment which are required and secondly, to eliminate the possibility of the ewes to be grouped in uneven sized groups, which may complicate the system.

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The wool properties and body weights of the indigenous Red Karaman and Karayaka sheep breeds of Turkey

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Introduction Sheep breeding has an important role in animal production in Turkey. The diversity of animal genetic resources of Turkey have not been fully appraised and some indigenous farm animal breeds are in danger of disappearing even before being scientifically defined. An equilibrium is needed between the genetic improvement studies and the conservation of the genetic resources Ozcan *et al* (1994). The greatest part of the wool produced by the indigenous sheep breeds in Turkey is of coarse and mixed type of wool and most of it is used in the hand woven authentic carpet production Kizilay (1986). This paper reports body weights and wool production characteristics of the indigenous Red Karaman and Karayaka sheep breeds.

Materials and methods The purebred samples of the Karayaka and Red Karaman genotypes, which were collected in the Altindere State Farm for the conservation of these breeds as genetic resources, were used in the study. Before shearing at the end of June in each year, wool samples were taken from the side (rib region) of each animal. Measurements for the various wool characteristics (fibre diameter, fibre length, fibre elasticity and breaking strength) were carried out at the Lalahan Animal Breeding Research Institute, Ankara. The body weights (after shearing) and greasy fleece weights of ewes were determined by the weighing of the ewes and the greasy fleece by a scale sensitive to 0.1 kg. The greasy fleece weight of the 1.5 year old ewes were not taken into consideration as their fleece represented 1.5 years not 1 year growth. In the analysis of the performance traits (body weights and greasy fleece weights), the following model was used: $Y_{ijkl} = \mu + G_i + A_j + V_k + AV_{jk} + e_{ijkl}$ Where Y_{ijkl} : Individual observation, μ : Overall mean, G_i : Effect of genotype (i = Karayaka and Red Karaman), A_j : Effect of age (j = 1.5, ..., 6.5), V_k : Effect of year (k = 2003 and 2004), AV_{jk} : Effect of age x year interaction, e_{ijkl} : Random error. The effects of the factors affecting the traits were studied using least square means (LSM) procedure.

Results The body weight results and greasy fleece weights of the ewes were presented in Table 1. The Red Karaman ewes were heavier than Karayaka ewes and the difference between the genotype groups was significant ($P < 0.01$). The body weight of the ewes got heavier as they grew older. The greasy fleece weight of the Karayaka ewes was higher than that of Red Karaman ewes ($P < 0.01$). The ewes gave heavier greasy fleece in the year 2003 and the ewes gave similar amount of greasy fleece at all ages but the greasy fleece weight was higher at 6.5 years of age. For the wool characteristics, the Karayaka and Red Karaman ewes displayed similar results for fibre diameter, fibre length and fibre elasticity. Only for breaking strength the wool of Karayaka ewes gave a higher result than that of Red Karaman ewes, the difference being statistically significant ($P < 0.01$).

Table 1 The LSM of the body weights and greasy fleece weights of the Red karaman and Karayaka ewes and the effect of factors affecting (EFA) on body weight and greasy fleece weights.

Factors	Body Weight					Greasy fleece weight				
	No	mean	s.e.d	EFA	s.e.d	No	mean	s.e.d	EFA	s.e.d
Genotype		**					**			
Karayaka	139	46.25 ^b	0.55	-5.73	0.35	115	2.54 ^a	0.06	0.14	0.04
Red Karaman	541	53.42 ^a	0.47	5.73	0.35	481	2.32 ^b	0.03	-0.14	0.04
Year		**					**			
2003	348	47.44 ^b	0.54	-2.52	0.33	309	2.56 ^a	0.05	0.17	0.03
2004	332	52.27 ^a	0.47	2.52	0.33	287	2.31 ^b	0.04	-0.17	0.03
Age		**					**			
1.5	84	41.37 ^c	0.83	-6.29	0.83	-	-	-	-	-
2.5	103	46.76 ^b	0.81	-2.02	0.77	103	2.32 ^b	0.07	-0.14	0.06
3.5	117	50.14 ^a	0.63	1.18	0.52	117	2.41 ^b	0.06	-0.08	0.05
4.5	129	50.56 ^a	0.67	1.72	0.56	129	2.43 ^b	0.06	-0.05	0.05
5.5	153	50.76 ^a	0.58	2.02	0.52	153	2.49 ^b	0.05	0.00	0.05
6.5	94	51.24 ^a	1.19	3.38	0.89	94	2.75 ^a	0.10	0.27	0.05
Age × Year		**					**			
Expected Mean	680	48.26	0.37			596	2.40	0.04		

** $P < 0.01$ In each of the subgroups, means followed by different superscripts differ significantly.

Conclusions In this study it was determined that the Karayaka ewes produced more fleece than Red Karaman ewes although their body weight was less and the wool of Karayaka ewes was stronger. The wool of both indigenous breeds was favourable to be used in the hand woven authentic carpet production.

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Study of the bone morphogenetic protein 15 (BMP15) mutation in Moghani sheep using RFLP method

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Introduction The Moghani is the main fat-tailed type of sheep in north-west of Iran and north-east of Turkey, where it is raised for meat, milk and carpet wool. In sheep, a wide range in litter size has been observed among different breeds and within breeds. Several genes have been recently identified that affect female fecundity in domesticated sheep such as *BMP15* gene which encodes bone morphogenetic protein 15 (Davis 2005 and Hanrahan *et al.*, 2004) and is a member of the transforming growth factor b (TGFb) super family (Galloway *et al.*, 2000). The identification of the *BMP15* mutation is important for the sheep industry and those involved in the study of mammalian fertility. In this study we investigated mutation of BMP15 gene in Moghani sheep in Iran.

Materials and methods The blood samples were taken from jugular vein of 108 Moghani sheep, and screened with the PCR-RFLP method for the BMP15 gene to study its mutation. DNA was isolated from whole sheep blood samples using DNA extraction Kit according to manufacturer instruction. PCR reactions were performed in a total volume of 50 µl, containing 50 ng of chromosomal DNA dissolved in TE buffer pH 8 and 5 pmol of each primer, 200 µM from the four dNTPs, 5 µl of 10X PCR buffer, 1.5 µl of 50 mM magnesium chloride, and 1U of *Taq*-Polymerase. The samples were subjected to 35 cycles of amplification in a TECHNE gene thermocycler. Each cycle consists of 1 min at 95°C, 1 min at 59°C and 45 sec at 72°C. PCR products were electrophoresed in 1% agarose gel and stained with ethidium bromide and photographed at UV exposure. The segment of BMP15 gene was amplified using PCR technique with the BM-f, BM-r primers (Hanrahan *et al.*, 2004).

Results PCR analysis for all samples showed the amplification of 141 bp segments of *BMP15* genes (Figure 1). Digestion of PCR products using *HinfI* enzyme for BMP15 gene were performed 106 bp and 35 bp bands for BM (+, +), 141 bp, 106 bp and 35 bands for BM (+, -) and 141 bp single band for BM (-, -) genotypes. Sequencing of the samples which were different in digestion band pattern revealed that in these samples a mutation occurred with transition of cytosine to thymine (C → T) in *BMP15* exon 2 (Figure 2).

Figure 2 The positions of the primers and restriction sites are shown.

Figure 1. PCR products and digestion results for *BMP15* gene. Lane 1: *BMP15* PCR product and lanes 2, 3 and 4: BM (+, +), BM (+, -) and BM (-, -) digestion results, respectively. Lane L: 100 bp molecular weight marker.

Conclusions Several researches showed that mutations in *BMP15* may cause either an increased ovulation rate or infertility. The mutation in BMP15 was detected in Moghani breed and future investigations are needed in order to determine the mutation effect of gene on fertility. So if such mutation has a positive effect on fertility we can select mutant male sheep and increase fertility in flock of sheep. Also PCR-RFLP test was found to be fast and useful as a tool for studying mutation.

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Evaluation of microwave irradiation effects on nutritive value of broom sorghum grain using an *in vitro* gas production technique

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Introduction In Iran various varieties of sorghum (*Sorghum vulgare*) are planted mainly for broom production but also for use in animal nutrition. There appears to be no documented research about nutritive value of microwave processed sorghum grain. With microwave irradiation, heat is generated throughout the material, leading to faster heating rates and shorter processing times compared to conventional heating, where heat is usually transferred from the surface to the interior (Fakhouri and Ramaswamy, 1993). In the *in vitro* gas production method for predicting nutritive value, the kinetics of fermentation can be studied on a single sample and therefore a relatively small amount of sample is required or a larger number of samples can be evaluated at any one time. Gas production measurement provides useful data on digestion kinetics of both soluble and insoluble fractions of feedstuffs (Getachew *et al.*, 1998). The purpose of this study was to evaluate effects of microwave irradiation on nutritive value of broom sorghum grain using the *in vitro* gas production technique.

Materials and methods The broom sorghum grain used in this study was obtained from Myaneh in Eastern Azarbaijan province in northwest Iran. Rumen fluid was provided by two fistulated sheep, fed twice daily a diet consisting of lucerne hay (400 g/kg) plus commercial sheep concentrate (600 g/kg). The rumen fluid was collected 2h after the morning feed and strained through four layers of cheesecloth. The dry matter (DM) content of the sorghum grain was determined (from duplicate 1g samples) and water was added to the sorghum grain to increase its moisture content to 300 g/kg. Three samples (100 g each) were subjected to microwave irradiation (Butane microwave BC380W) at a power of 900w for 3, 5 and 7 min. Gas production was measured using the method of Fedorak and Hudey (1983). Triplicate samples of ground and dried broom sorghum grain (approximately 300mg) were weighed and placed in 50 ml capacity serum bottles; they were then incubated in 20 ml of Macdougall buffered rumen fluid (buffer: rumen fluid, 2:1, v/v) for 48h. Gas production was recorded at 2, 4, 6, 8, 12, 16, 24, 36 and 48 h of incubation using a water replacement assay. The data at the different times was analysed using completely randomised design by the GLM procedure of SAS Institute Inc (2002).

Results The gas production data of untreated and microwave-treated sorghum are shown in Table 1. *In vitro* gas production of sorghum grain was significantly increased by microwave irradiation ($P<0.05$). There were no differences for gas production between 5 and 7 min microwave treated sorghum ($P>0.05$).

Table 1 The effect of microwave irradiation of sorghum grain on *in vitro* gas production (ml/gDM)

Time	Untreated sorghum grain	Microwave irradiated sorghum grain			s.e
		3 min	5 min	7 min	
2h	9.4 ^b	10.2 ^b	14.7 ^a	14.5 ^a	1.23
4h	21.7 ^c	23.5 ^{bc}	30.2 ^a	28.8 ^{ab}	1.74
6h	34.9 ^b	35.4 ^b	44.5 ^a	42.7 ^a	2.04
8h	45.6 ^b	45.2 ^b	56.4 ^a	53.8 ^a	2.12
12h	63.6 ^b	64.2 ^b	74.8 ^a	75.2 ^a	2.64
16h	86.6 ^c	91.6 ^{bc}	104.5 ^{ab}	110.6 ^a	4.07
24h	132.8 ^b	145.3 ^{ab}	153.9 ^a	162.6 ^a	5.40
36h	217.8 ^a	237.0 ^a	214.2 ^a	236.3 ^a	7.07
48h	272.0 ^{ab}	285.8 ^a	260.1 ^b	282.2 ^{ab}	7.29

^{ab}The means within a rows without common letter differ ($P<0.05$).

Conclusions The current study indicated that microwave processing increased gas production of sorghum grain, probably because linkages between the protein matrix and the starch granule were disrupted. It seems that microwave treatment increases starch availability and gelatinisation. Further study is needed to determine effects of different microwave powers and times on sorghum *in vitro* gas production.

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Disappearance of dry matter and neutral detergent fibre (NDF) of sunflower meal treated with sodium hydroxide or formaldehyde by isolated mixed rumen bacteria using *in vitro* culture

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Introduction Cellulolytic bacteria, such as *Ruminococcus albus*, *R. flavefaciens* and *Fibrobacter succinogenes* are major micro-organisms responsible for ruminal digestion of plant cell walls ingested by the animal due to their numerical predominance and metabolic diversity (Cheng *et al.*, 1991). It has been proposed that sodium hydroxide might breakdown hemicelluloses, expose the cellulose to microbial attachment and improve digestibility (Chen *et al.*, 2006). The objective of this experiment was to estimate the disappearance of dry matter (DM) and neutral detergent fibre (NDF) of sunflower meal (25 g fat/kg DM; SM) as untreated or treated with formaldehyde (3 g/kg DM) or sodium hydroxide (40 g/kg DM) using *in vitro* culture with isolated mixed rumen bacteria.

Materials and methods Rumen fluid was collected from four fistulated sheep which fed 250 g concentrate, 550 g lucerne hay and 200 g wheat straw, then centrifuged (1000 rpm, 10 min). Supernatant was used to grow bacteria in bacteria medium containing fungicides (benomyle: 500 ppm/ml of medium and metalaxyle: 10 mg/ml of medium) under anaerobic conditions at 39 °C for 24 h. These isolates were then used as a source of inoculum for culturing bacteria in a serum bottle containing 45 ml of culture medium of bacteria (Galdwell and Bryant, 1966) and 1g of SM as untreated or treated with formaldehyde (3 g/kg DM) or sodium hydroxide (40 g/kg DM) under anaerobic conditions (using three times subculture), at 39 °C in an incubator for 12, 24, 48, 72 and 96 h. Three replicates were run per treatment per time. The residual substrates of each bottle were then filtered and used to determine the DM and NDF concentrations. Data for DM and NDF disappearance in different times were analysed as a completely randomised design using the General Linear Model (GLM) procedure of SAS (1990). Duncan's multiple range test was used to compare the means at $P < 0.05$.

Results Disappearance of DM and NDF of samples using isolated mixed rumen bacteria culture are given in Table 1. Disappearance rate of DM and NDF of sodium hydroxide treated samples was markedly higher than the other samples, in each incubation time. Formaldehyde caused a decrease in the disappearance of DM and NDF of SM using rumen mixed bacterial *in vitro* culture.

Table 1 Dry matter (DM) and neutral detergent fibre (NDF) disappearance of sodium hydroxide or formaldehyde treated sunflower meal using isolated rumen bacteria *in vitro* culture

IT (h)	DM disappearance (g /100g)					NDF disappearance (g/100 g)				
	USM	SHSM	FSM	s.e.m	P	USM	SHSM	FSM	s.e.m	P
12	36.3 ^b	42.0 ^a	31.2 ^c	0.77	< 0.01	10.1 ^b	14.5 ^a	8.3 ^c	0.51	< 0.01
24	42.6 ^b	47.1 ^a	33.4 ^c	0.65	< 0.01	19.5 ^b	21.3 ^a	15.9 ^c	0.72	< 0.01
48	51.1 ^b	58.2 ^a	44.2 ^c	0.79	< 0.01	26.5 ^b	29.6 ^a	23.3 ^c	0.80	< 0.01
72	59.2 ^b	67.1 ^a	54.1 ^c	0.87	< 0.01	28.2 ^b	31.0 ^a	24.4 ^c	0.84	< 0.01
96	57.3 ^b	62.3 ^a	56.3 ^b	0.62	< 0.01	27.7 ^b	30.6 ^a	23.7 ^b	0.72	< 0.01

IT: incubation time; USM: untreated sunflower meal (SM); SHSM: 40 g NaOH /kg DM of SM; FSM: 3 g formaldehyde /kg DM of SM; s.e.m: standard error of mean; abc: means with different letters within each row differed significantly.

Conclusions *In vitro* bacterial digestion of feed samples used in the present experiment was influenced by chemical compounds of sodium hydroxide or formaldehyde. Sodium hydroxide caused to increase the bacteria digestion of DM and NDF, while formaldehyde decreased the digestion using *in vitro* culture. Results of the present study confirmed previous results which used an alkaline solution to increase the disappearance rate of DM and NDF. Gould (1984) reported that the dilute solutions of alkaline react with lignocelluloses to yield partially de-lignified products that are highly susceptible to enzymatic and microbial attack. Bas *et al.* (1989) reported that the DM and NDF digestion of straw treated with an alkali was greater than untreated straw. Results of the present study indicated that the potential improvement in DM and NDF digestion of sunflower meal could be resulted from the use of sodium hydroxide.

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Antinutritional factors, ruminal degradation and *in vitro* protein digestibility of gamma irradiated full-fat soybean

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Introduction Whole soybean is used as a high energy-protein supplement for dairy cows, but contains various antinutritional factors and the protein is highly degradable by rumen microbes. Roasting and extrusion are very popular ways of feeding soybeans to dairy cows but these processing methods may adversely affect the protein digestibility and lysine availability of the final product in the small intestine. Gamma irradiation has been recognized as a reliable and safe method for improving the nutritional value and inactivation or removal of certain antinutritional factors in foods and feeds (Siddhuraju *et al.*, 2002). As far as we know, information about the effects of γ -irradiation on ruminal crude protein (CP) degradation of full-fat soybean in the literature is scarce. The aim of this study was to evaluate the effects of gamma irradiation (γ -irradiation) on nutritional value and antinutritional factors of soybean (SB) for ruminants.

Materials and methods The DM of soybean was determined by oven drying 1g sample in duplicate. Then sufficient water was added to sample to increase the moisture content of soybean to 250 g/kg. Three paper packages of samples were irradiated in a gamma cell for total doses of 15, 30 and 45 kGy in the presence of air. Nylon bags (10 cm $\times$ 20 cm; 45 μ m pore size) were filled with approximately 6 g sample of untreated or γ -irradiated (size: bag surface area of 15 mg/cm²) and suspended in the rumen of three ruminally fistulated bulls (416 $\pm$ 18 kg) for up to 48 h and resulting data were fitted to nonlinear degradation model to calculate degradation parameters of DM and CP according to the equation of Ørskov and McDonald (1979). Digestibility of rumen undegraded CP was estimated using the three-step *in vitro* procedure of Calsamiglia and Stern (1995). The nitrogen in feeds and residues after rumen and *in vitro* incubation was determined according to AOAC (Method 984.13; AOAC, 1995). Degradability data were analyzed as completely randomized block design according to the general linear models procedure of SAS (1996) with the following statistical model of $Y_{ijk} = \mu + T_i + B_j + e_{ijk}$. Where Y_{ijk} is dependent variable, μ is overall mean, T_i is γ -irradiation effect, B_j is animal effect, and e_{ijk} is residual error, assumed normally and independently distributed. Means were compared by Least Squares Means.

Results The antinutritional, CP degradability and *in vitro* CP digestibility contents of the SB are listed in Table 1. Results showed that phytic acid and trypsin inhibitor activity in γ -irradiated SB were decreased ($P < 0.01$). From *in sacco* results, γ -irradiation decreased ($P < 0.05$) the washout fractions (a) of CP at doses of 30 and 45 kGy but increased ($P < 0.05$) the potentially degradable fractions. Gamma irradiation decreased ($P < 0.05$) effective degradability of CP at rumen outflow rate of 0.05 h⁻¹. However digestibility of ruminally undegraded CP of irradiated SB improved ($P < 0.05$) at doses of 30 and 45 kGy was (table 1).

Table1 Antinutritional factors, rumen protein degradability and *in vitro* protein digestibility of the treatments

Parameters	Untreated Soybean	Gamma-irradiated Soybean			S.E.M
		15kGy	30kGy	45kGy	
Rumen degradation parameters crud protein					
a (the washout fraction, g/kg)	346 ^a	336 ^a	281 ^b	216 ^c	14.5
b (the potentially degradable fraction, g/kg)	662 ^c	665 ^c	716 ^b	761 ^a	12.0
c (the constant rate of disappearance of b, h ⁻¹)	0.103 ^a	0.081 ^b	0.061 ^c	0.046 ^d	0.0034
Effective rumen degradation (0.05 h ⁻¹ , g/kg)	792 ^a	749 ^b	678 ^c	582 ^d	11.2
<i>In vitro</i> crude protein digestibility (g/kg)	570 ^a	585 ^a	638 ^b	730 ^c	9.5
Phytic acid (mg/100g)	234 ^a	94.3 ^b	0.0 ^c	0.0 ^c	8.9
Trypsin inhibitor factor (TIU /g)*	32.1 ^a	26.2 ^b	14.3 ^c	11.7 ^d	3.3

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

* Trypsin inhibitor unit.

Conclusion It was concluded that, γ -irradiation was effective in decreasing ruminal protein degradability, phytic acid content, trypsin inhibitor activity and increasing of *in vitro* CP digestibility of full-fat soybeans. Consequently, results in this study suggest that γ -irradiation can be used to improve nutritive value of full-fat soybeans at doses higher than 15 kGy.

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The chemical composition, *in vitro* and *in situ* digestibility of whole crop canola silage mixed with different by-products

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Introduction Canola (*Brassica napus* L.) recently has received consideration as a forage source for livestock (Lancaster *et al.*, 1990). Canola is reasonably widely adapted and performs well in semiarid countries such as Iran. In addition to its low water requirements and high growth rate, its cultivation season is well matched with environmental condition in Iran. Canola could not reach to stage of blossom in some climates such as cold climate, so farmers could mow and making silage for animal nutrition. Application of silage additives is the most important factor in improving silage quality (Muck, 1993). The main objective of this study was to evaluate the effect of some additives on fermentation parameters, *in vitro* and *in situ* dry matter digestibility of experimental whole canola silage.

Materials and methods Whole crop canola (WCC) was ensiled in 10 Kg plastic containers using 10% (on DM basis of canola forage) different additives in a completely randomized design with three replicates. Treatments were, T1) WCC + molasses, T2) WCC + beet pulp, and T3) WCC + citrus pulp. Canola (hyula 308), were seeded in October and mow in June. Forages were chopped in size of 1-2cm, and preserved in nylon bags for 60 days in a cool and dry place. After this period, silages were dried in 60 C for 48h in oven and grounded through a 1mm mill screen, then analysed for pH, DM, OM, CP, NDF and ADF contents in accordance to the AOAC (1990) methods. The *in vitro* Dry Matter and Organic Matter Digestibility (IVDMD and IVOMD) of treated silages were determined using *in vitro* 2-step digestion technique, according to the Tilley and Terry method. The *in situ* ruminal disappearances of silages were measured by using ruminal nylon bags (12×17 cm) with three replicates. The ingredients were dried and grounded through a 2 mm mill screen. The bags were filled with 5g of samples and put in to the rumen of three ruminal fistulated sheeps. The bags were removed at 24, 30, 48 and 72h after the start of incubation and each bag was washed immediately with tap water until colour disappeared. For the t_0 incubation time, the bags were just washed in the water. Data were analyzed by analysis of variance using the GLM procedure of SAS (2002) in a completely randomized design. The following model was fitted: $Y_{ijk} = \mu + T_i + e_{ij}$, where Y_{ijk} = dependant variable, μ = overall mean, T_i = treatment effect and e_{ij} was residual error. Differences between means were compared with the Duncan Test.

Results Table 1 shows adding by-products could decrease pH of silages, specially molasses and beet pulp ($P < 0.05$). The NDF content of silages were decreased significantly by adding other feedstuffs in it ($P < 0.05$). IVDMD of WCC + molasses, beet pulp or citrus pulp silages were higher than WCC silage ($P < 0.05$). The amount of IVDMD of treatment containing citrus pulp was highest than others ($P < 0.05$); it seems the high degradation rate of citrus pulp is responsible for it, although high ash content of citrus pulp cause to decrease IVOMD of the T3 in compare to the T2. The *in situ* disappearance of treatments in different times showed that control had lowest DM degradability in the rumen, but citrus pulp + WCC silage had more soluble and degradable dry matter than others (Fig. 1). The NDF disappearance of control and T3 were lowest and highest among silages, respectively (Table 1).

Table1 The Effects of treatments on chemical value of canola silage.

Item	Treatments				SEM
	Control	1	2	3	
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
EE	1.3 ^{ab}	1 ^b	2 ^{ab}	2.3 ^a	0.22
Ash	9.95 ^b	9.4 ^c	9.4 ^c	11.6 ^a	0.00
IVDMD	56.6 ^c	58.5 ^b	59.0 ^b	65.5 ^a	0.06
IVOMD	65.6 ^b	66.5 ^b	75.8 ^a	69.7 ^b	0.01
NDFD-30h	0.597 ^c	0.635 ^b	0.651 ^b	0.707 ^a	0.001

a, b, c, d In the same row, the numbers with different letters are different ($P < 0.05$).

Figure 1 The *in situ* DM disappearance of treated silages in different hours.

Conclusion According to these results it seems by applying some by-products, which are good sources of soluble carbohydrate, in canola silage, the chemical and nutritional value of the canola mix silage could be improved than canola silage.

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Effects of *Saccharomyces cerevisiae* on the nutritive value of lucerne hay assessed with the *in vitro* gas production technique

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Introduction Yeast culture (*Saccharomyces cerevisiae*) has been widely used as a dietary supplement in ruminants. *Saccharomyces cerevisiae* supplementation in ruminant diets can increase dry matter intake, production performance, cellulose degradation, and nutrient digestibility (Callaway and Martin, 1997). *In vitro* techniques are routinely used to evaluate the nutritional quality of feeds because of their convenience, adaptability and efficiency. The *in vitro* gas production technique is an accurate and fast method to evaluate the nutritive value of feeds and it can be a good alternative method instead of *in vivo* trials to evaluate commercial feed additives. The objective of this study was to evaluate the effects of different levels of inclusion of *Saccharomyces cerevisiae* on the nutritive value of lucerne hay using the *in vitro* gas production technique.

Materials and methods Gas production in each serum bottle (vial) was measured using a water displacement apparatus (Fedorak and Hudey, 1983). Two fistulated sheep (39±0.5kg) were used as donors of rumen liquor. Sheep daily diets contained 400 g/kg forage plus 600 g/kg concentrate. Rumen liquor was obtained 2 hours after morning meals and strained through four layers of cheesecloth into a pre-warmed flask. The treatment contained 0, 2.5, 5 and 7.5 g yeast *Saccharomyces cerevisiae* (Sc) per kg DM lucerne hay. Dried (96°C oven for 48 h) and milled (2 mm screen) lucerne hay (300 mg) was accurately weighed and placed into 50 ml serum bottles in triplicate then prepared treatments were added to bottles according to the above mentioned rate. Rumen fluid buffered with McDougal buffer (ratio of buffer to rumen fluid was 2:1) was added to each bottle (20ml). Gas production was recorded after 2, 4, 6, 8, 12, 16, 24, 36, 48 h of incubation. Total gas values were corrected for blank incubations. Gas production profiles were fitted to the curve $Y = A(1 - e^{-ct})$ where Y is the volume of gas produced (ml/g DM) at time t, A is asymptotic gas volume, c is the gas production rate and t is the incubation time (h). *In vitro* digestibility of organic matter (OMD) and metabolisable energy (ME) of treatments were calculated by the equation of Menke and Steingass (1988). Data were analyzed as a complete randomized design using the GLM procedure of SAS Institute Inc (2002).

Results Gas production parameters of treatments are shown in Table 1. Gas production volume of lucerne hay in most of the incubation times were affected by different levels of yeast culture and differences between them were significant ($p < 0.01$). Potential gas production and gas production rate were significantly different ($p < 0.01$) as well. Treatment of lucerne hay with 7.5 g/kg DM yeast culture produced the greatest volume of gas, potential gas production and gas production rate. *In vitro* digestibility of organic matter and ME were affected by yeast culture and treatment without *Saccharomyces cerevisiae* was lower than the treatments with *Saccharomyces cerevisiae* ($p < 0.01$).

Table 1 Gas production parameters, OMD and ME of treatments *

	Gas production (ml/ g DM)			Gas production parameters			
	8h	16h	48h	A(ml/g DM)	c (/h)	OMD (g/kg OM)	ME(MJ/kg DM)
Control	147.53 ^b	221.97 ^b	278.77 ^b	278.67 ^d	0.1134 ^b	154.8 ^b	23.6 ^b
Sc2.5 g/kg DM	140.97 ^b	225.40 ^b	288.43 ^{ab}	297.08 ^b	0.0997 ^c	167.65 ^{ab}	25.57 ^{ab}
Sc 5 g/kg DM	146.77 ^b	222.73 ^b	289.53 ^{ab}	286.74 ^c	0.0975 ^d	164.19 ^b	25.05 ^{ab}
Sc7.5 g/kg DM	177.37 ^a	251.8 ^a	318.93 ^a	309.28 ^a	0.1185 ^a	186.06 ^a	28.38 ^a
SEM	3.77	5.11	7.94	1.005	0.00024	4.01	0.61

* The means within columns without a common letter differ ($p < 0.01$).

Conclusions It was concluded that *in vitro* gas production parameters, OMD and ME of lucerne hay were improved with the addition of yeast *Saccharomyces cerevisiae* so yeast culture had potential of improving rumen fermentation characteristics. An addition of 7.5 g Sc per kg DM lucerne hay have enough ability to improv rumen fermentation characteristics significantly. Sullivan and Martin (1999) found *Saccharomyces cerevisiae* culture filtrate stimulated the initial rate of cellulose degradation. This may explain the increase in the rate of gas production by the addition of yeast in the current study. Also, the *in vitro* gas production technique was able to detect effects of feed additives on rumen fermentation.

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Effect of adding different levels of probiotic on *in vitro* gas production

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Introduction Probiotics present an attractive alternative to the use of chemical and hormonal promoters in the livestock growth production industry. Preparations that contain micro-organisms have been safely used for many years and are generally accepted by both the farmer and the final consumer. *Saccharomyces cerevisiae* supplementation in ruminant diets can increase DMI, production performance, cellulose degradation, and nutrient digestibility (Callaway and Martin, 1997). The gas measuring technique has been widely used for the evaluation of nutritive value of feeds. Gas measurement provides 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 sample is required or a larger number of samples can be evaluated at time. The purpose of this study was to study effect of adding different levels of *Saccharomyces cerevisiae* on *in vitro* gas production from a dried grape by-product.

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 in each serum bottles (vial) was measured using a water displacement apparatus (Fedorak and Hudey, 1983). Approximately 300 mg of dried and ground (2mm) dried grape by-product sample was weighed and placed into serum bottles in triplicate. The treatment contained 0, 2.5, 5 and 7.5 g yeast *Saccharomyces cerevisiae* (Sc) per kg of dried grape by-product based on DM, respectively. Buffered rumen fluid with McDougal buffer (20ml, ratio of buffer to rumen fluid was 2:1) was pipetted into each serum bottle (McDougal 1948). The gas production was recorded after 2, 4, 6, 8, 12, 16, 24, 36, and 48 h of incubation. Total gas values were corrected for the blank incubation, and reported gas values are expressed in ml per 1 g of DM. The data at the different times was analyzed using completely randomized design by the GLM procedure of SAS Institute Inc (2002). The organic matter digestibility, ME and NE_L contents of forages were estimated by the method of Menke and Steingass (1988).

Results Total gas production volume (ml/ g DM) by incubation times are shown in Table 1. At the first incubation time (2h), the control treatment had the highest gas production volume but by 4 h the gas production volume in experimental treatments (treatments with Sc) had increased and the all treatments had the same gas production volume ($p < 0.05$). At 6 h and beyond, the treatments containing Sc, had the highest and control treatment had lowest gas production volume ($p < 0.05$). Adding Sc to treatments increased the estimated amounts of OMD, ME and NE_L. The pattern of *in vitro* gas production in treatments is shown in figure 1. It is observed that adding *Saccharomyces cerevisiae* increased *in vitro* gas production and a maximum stimulation could be obtained with the lowest addition concentration of Sc.

Table 1 Total gas production volume (ml/ g DM) in incubation times*

Treatments	Items		
	OMD (%)	ME _L (MJ/kg DM)	NE _L (MJ/kg DM)
Control	55.41	8.25	4.68
Sc 2.5 g/kg DM	68.05	9.40	5.52
Sc 5 g/kg DM	68.65	9.48	5.58
Sc 7.5 g/kg DM	68.79	9.50	5.60

Conclusions It was concluded that *in vitro* gas production parameters, OMD, ME and NE_L of dried grape by-product were improved by the addition of yeast *Saccharomyces cerevisiae*, and that the yeast culture had the potential to improve rumen fermentation characteristics.

Acknowledgement This study was funded by University of Tabriz, Iran.

Figure 1 Pattern of *in vitro* gas production in treatments.

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The effect of silver nano-particles (Nanosilver) on *in vitro* gas production of barley grain and lucerne hay

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Introduction It is generally believed that heavy metals react with proteins by combining the thiol (-SH) groups, which leads to the inactivation of the proteins (Cho *et al.*, 2005). Silver nano-particles (Ag-NPs) are being used vastly as a strong anti-germ product. In general, Ag ions, which have antimicrobial activity, are used as an antibacterial agent. The antibacterial activity of Ag ions inhibits intracellular enzyme activity (Cho *et al.*, 2005). Therefore, the other possibility can be considered that remaining Ag ions in Ag-NPs solution or dissolved Ag ions might affect bacterial growth. Recently, dairy farmers have tended to apply this anti-bacterial for sanitization of the environment (as a disinfectant material) of animals. However, the environmental residual effect of this anti-bacterial on ruminant microbial activity is still questionable. In the present study, a gas production technique was applied in order to determine the effect of silver nano-particles on *in vitro* gas production of barley grain and lucerne hay.

Materials and methods The gas production technique was conducted according to Menke and Steingass (1988) procedure. Rumen fluid was taken from three sheep before the morning feeding and was passed through 4 layers of cheese cloth. Adding of CO₂ gas was continued after the rumen fluid was poured in the syringes. The applied solutions were: Main element solution (5.7 g Na₂HPO₄ + 6.2 g KHPo₄ + 0.6 g MgSO₄ × 7H₂O) made up to 1 L with distilled water. Trace element solution (13.2 g CaCl₂ × 2H₂O + 10.0 g MnCl₂ × 4H₂O + 1g CoCl₂ × 6 H₂O + 0.8 g FeCl₂ × 6H₂O) made up to 100 ml with distilled water. Buffer solution (35 g NaHCO₃ + 4 g (NH₄)HCO₃) made up to 1 L with distilled water. Resazurin solution (100 mg resazurin) was made up to 100 ml with distilled water. Reduction solution: First 2 ml 1N-NaOH and then 285 mg Na₂S × 7H₂O were added to 47.5 ml distilled water. Solutions were mixed up in the following order: 474 ml distilled water, 0.12 ml trace element solution, 237 ml buffer solution, 237 ml main element solution and 1.22 ml resazurin solution. Samples (barley grain or lucerne hay) were milled to pass a 2 mm screen. 0.3 g of each sample was placed in gas production syringes. Then, the nanosilver solutions which were prepared in three different dilutes (40,100 and 160 ppm) were added into the syringes (1 ml per each syringe). The treatments were: lucerne hay, lucerne hay + 40 ppm nanosilver, lucerne hay + 100 ppm nanosilver, lucerne hay + 160 ppm nanosilver, barley grain and barley grain + 100 ppm nanosilver. Four replications were run per each treatment. The ratio of rumen fluid to buffer solution was 1:2 (10 ml rumen fluid and 20 ml buffer solution per each syringe). Then, the syringes were incubated in a 39 °C pre-heated water bath. The amount of produced gas was determined at 2, 4, 6, 8, 12, 24, 48 and 72 hours after the incubation. Data were analyzed using feed-plot program based on the model of $P = b(1 - e^{-ct})$, where P = amount of produced gas in time, b = gas production from fermentable fraction and c = fractional constant rate of gas production.

Results Gas production coefficients are indicated in Table 1. Adding nanosilver to the syringes had no significant effect on the amount of produced gas for either lucerne hay and barley grain.

Table1 The effect of nanosilver on gas production coefficients of lucerne hay and barley grain (mean ± SE)

Item	Gas production coefficients		
	Gas production from fermentable fraction	Fractional constant rate of gas production	Coefficient of determination (R ²)
Lucerne hay	43.6 ± 2.07	0.12 ± 0.010	0.96
Lucerne hay + nanosilver (40 ppm)	45.8 ± 5.10	0.10 ± 0.020	0.83
Lucerne hay + nanosilver (100 ppm)	43.0 ± 2.80	0.10 ± 0.010	0.95
Lucerne hay + nanosilver (160 ppm)	46.4 ± 3.60	0.10 ± 0.010	0.91
Barley grain	59.2 ± 6.60	0.05 ± 0.020	0.84
Barley grain + nanosilver (100 ppm)	77.7 ± 2.10	0.05 ± 0.005	0.98

Conclusions Results of the present study indicated that there was no inhibitory effect of nanosilver on *in vitro* gas production for either lucerne hay or barley grain. Therefore, it seems there was no effect of the nanosilver on *in vitro* microbial activity as observed from the gas production coefficients. However, further research is needed to evaluate the impact of this anti-bacterial product on *in vitro* microbial growth rate and *in vivo* conditions.

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In situ* dry matter degradability coefficients of whole crop barley silage treated with *Lactobacillus plantarum* or mixed with *Pediococcus pentosaceus* plus *Propionibacter freudenreichii

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Introduction Microbial inoculants are applied to forage at the time of ensiling to accelerate the decline of pH during the initial stage of silage fermentation, to preserve plant carbohydrates through homofermentation, and to preserve plant protein by decreasing proteolysis and deamination. Thus, inoculated silages are expected to improve animal performance. Whole crop barley has a low buffering capacity and abundant fermentable carbohydrates and is considered relatively easy to ensile. Results of previous experiments indicated that lactic acid bacteria-based inoculants have the potential to improve the ensilage of whole crop barley (Kung and Ranjit, 2001). The objective of the present study was to determine the chemical composition and *in situ* dry matter degradability of whole crop barley silage treated with one of two different types of inoculants (*Lactobacillus plantarum* or mixed with *Pediococcus pentosaceus* plus *propionibacter freudenreichii* as provide 1×10^5 CFU of lactic acid producing bacteria plus 1×10^4 CFU *propionibacterium* per g of DM).

Materials and methods Whole crop barley was harvested (about 35% DM), chopped, and then ensiled as untreated or treated with different inoculants including *Lactobacillus plantarum* (8×10^{10} CFU (LP8) or 16×10^{10} CFU (LP16) per g of DM) or mixed with *Pediococcus pentosaceus* plus *propionibacter freudenreichii* (5.5×10^{10} CFU (PP5.5) or 11×10^{10} CFU (PP11) per g of DM) for 30 days ($n = 4$). Standard procedures were used to determine the chemical composition of the samples. The pH of the aqueous silage extract was determined using a pH meter (Metrohm 691, Swiss). $\text{NH}_3\text{-N}$ concentration was determined in acidified silage extract (5 ml of the extract + 5 ml of 0.2 M HCl) using a distillation method (Kjeltec 2300 Autoanalyzer, FossTecator AB, Hoganas, Sweden). Rumen degradability of the silages was determined using an *in situ* procedure (Fathi Nasri *et al.*, 2006). Four sheep (44 ± 5 kg liveweight) fitted with rumen cannulae were used. The bags (10×12 cm) were made of polyester nylon cloth with a pore size of 52 μm . About 5 g DM of each sample was placed in each bag, and four bags per treatment were incubated for each time (0.0, 2, 4, 8, 16, 24, 48, 72, 96 h). The equation of $P = a + b(1 - e^{-ct})$ was applied to determine the coefficients (a = quickly degradable fraction, b = slowly degradable fraction, c = fractional degradation rate constant).

Results Chemical composition and the degradable coefficients of DM of whole crop barley silages are shown in the Tables 1 and 2, respectively. The data suggest that the inoculants tended to decrease pH and $\text{NH}_3\text{-N}$ concentration and increase NDF content of the treated compared with untreated silage. Treated silages had a higher slowly degraded fraction compared with untreated samples.

Table 1 Chemical composition of whole crop barley silage treated with *Lactobacillus plantarum* or mixed with *Pediococcus pentosaceus* plus *propionibacter freudenreichii*

Item	untreated	<i>Lactobacillus plantarum</i>		Mixed bacteria		s.e.m.	P - value
		LP8	LP16	PP5.5	PP11		
pH	4.07 ^a	3.69 ^c	3.97 ^{ab}	3.98 ^{ab}	3.88 ^b	0.04	0.01
CP (g/kg DM)	7.98	7.87	7.91	7.86	8.02	0.08	0.21
$\text{NH}_3\text{-N}$ (mg/dl)	9.10 ^a	8.47 ^b	8.62 ^a	8.79 ^a	8.47 ^{ab}	0.35	0.02
NDF (g/kg DM)	554 ^c	581 ^b	627 ^a	542 ^c	617 ^a	6.11	0.01

^{a,b,c}Means with different superscript letters in the same row differed ($p < 0.05$)

Table 2 Dry matter degradable coefficients of whole crop barley silage treated with *Lactobacillus plantarum* or mixed with *Pediococcus pentosaceus* plus *propionibacter freudenreichii*

Degradation coefficient	untreated	<i>Lactobacillus plantarum</i>		Mixed bacteria	
		LP8	LP16	PP5.5	PP11
a	$0.37^a \pm 0.02$	$0.36^{ab} \pm 0.02$	$0.33^b \pm 0.01$	$0.34^{ab} \pm 0.02$	$0.33^b \pm 0.02$
b	$0.44^b \pm 0.03$	$0.48^{ab} \pm 0.02$	$0.49^a \pm 0.01$	$0.47^{ab} \pm 0.02$	$0.49^a \pm 0.02$
c	0.04 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.05 ± 0.01

a = rapidly degradable, b = slowly degradable, c = fractional degradation rate constant

Mixed bacteria: *Lactobacillus plantarum* plus with *Pediococcus pentosaceus* and *propionibacter freudenreichii*

Conclusions The results of the present study indicate that whole crop barley silage treated with *L. plantarum* has a lower pH and $\text{NH}_3\text{-N}$ concentration compared with untreated silage. This finding supports the results of Kung and Ranjit (2001). Therefore, we conclude that *L. plantarum* prevented proteolysis during ensiling. In addition, the increase in both b and c coefficients of DM degradation might help to enhance the digestibility and dry matter intake in ruminants.

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Evaluation of nutritional value in some tropical by-products using the *in vitro* gas production technique

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Introduction Anaerobic digestion of carbohydrates by ruminal microbes produces short chain fatty acids (SCFA), CO₂, CH₄, and traces of H₂; hence, measurement of gas production *in vitro* can be used to study the rate and extent of digestion of feedstuffs (Hungate, 1966). When a feedstuff is incubated with buffered rumen fluid *in vitro*, the carbohydrates are fermented to SCFA, gases mainly CO₂ and CH₄ and microbial cells. Gas production is basically the result of fermentation of carbohydrates to acetate, propionate and butyrate (Wolin, 1960; Beuvink and Spoelstra, 1992; Blummel and Ørskov, 1993). High correlations between gas production and NDF disappearance, $r^2 = 0.99$ (Pell and Schofield, 1993) or gas production and DM disappearance, $r^2 = 0.95$ (Prasad *et al.*, 1994) have been reported. *In vitro* techniques that estimate digestion kinetics indirectly by measuring gas production are a more viable option than other *in vitro* methods. Gas production technology allows for a more usable collection of digestion kinetics data and has allowed for a growing body of knowledge that is directly applicable to the feeding programs that are in daily practical field use. The range of data that can be acquired is broad and will no doubt grow over time. One of the most challenging problems associated with using gas production methods is that the amount of gas produced varies with different molar proportions of SCFA. For example, a higher propionate concentration is associated with lower gas production because an extra carbon atom in propionate would otherwise have appeared as CO₂ (Wolin, 1960). The object of this study was to evaluate the nutritional quality of noodle waste (NW), tomato pomace (TP) and apple pomace (AP) using the gas production technique.

Materials and methods Rumen fluid was collected approximately 2 h after morning feeding from two cannulated sheep consuming 400 g lucerne hay, 200 g barley and 200 g soybean meal. Rumen fluid was immediately squeezed through four layers of cheesecloth and was transported to the laboratory in a sealed thermos. The resulting rumen fluid was purged with deoxygenated CO₂ before use as the inoculum. Gas production in each serum bottles (vial) was measured using a water displacement apparatus (Fedorak and Hruday, 1983). Approximately 300 mg of dried and ground (2 mm) sample was weighed and placed in serum bottles. Buffered rumen fluid with McDougal buffer (20ml, ratio of buffer to rumen fluid was 2:1) was pipetted into each serum bottle in triplicate (McDougal, 1948). The gas production was recorded after 2, 4, 6, 8, 12, 16, 24, 36, and 48 h of incubation. Total gas values were corrected for the blank incubation. The gas production profiles in triplicate fitted with the equation of $Y = A(1 - e^{-ct})$ where Y is the volume of gas produced (ml/g DM) at time t, A is the asymptotic volume of gas produced (from the soluble and insoluble fraction), c is the gas production rate and t is the incubation time (h). The ME and OMD contents of feedstuffs were calculated using equations of Menke *et al.* (1979). Samples of feeds were collected from food factories in the country of Tabriz. The chemical composition of feeds was determined using the methods recommended by AOAC (2005).

Results The chemical compositions of feedstuffs are presented in Table 2. Gas production characteristics of feedstuffs are shown in Table 1. The fraction A for AP, TP and NW was 288, 199 and 266 ml/g DM, respectively, and there was a significant difference between feedstuffs. The gas production rate was 0.084, 0.09 and 0.081, respectively. The ME was 9.7, 8.18 and 8.53 MJ/kg DM, respectively. The OMD was 64.36%, 56.05% and 57.01%, respectively. The potential of gas production (A) for AP was more than other feedstuffs ($P < 0.05$), and the fraction c for TP was higher than other feedstuffs.

Table 1 Gas production characteristics*

Feeds	Items			
	A ¹	c ²	ME(MJ/kg DM)	OMD(%)
Apple pomace	288 ^a	0.084 ^b	9.7	64.4
Tomato pomace	199 ^c	0.090 ^a	8.2	56.1
Noodle waste	266 ^b	0.081 ^c	8.5	57.0
MSE	0.0004	0.00006	-	-

* The means within a column without common letter differ ($p < 0.05$).

1- The potential of gas production (ml/g DM)

2- The rate of gas production (/h)

Table 2 The chemical composition of feeds (%)

Feeds	Items				
	DM	CP	EE	ADF	ASH
AP	92.3	5.3	3.7	28	2.2
TP	96.5	21.6	6.9	58.7	12.2
NW	90.8	11.3	0.7	-	1.2

Conclusion These results indicated that there are differences in the degradability characteristics of these feedstuffs, which may need to be considered when formulating diets for ruminant animals.

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Evaluation of nutritive value of white mulberry (*Morus alba*) fruits waste

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Introduction The mulberry belongs to the genus *Morus* of the family Moraceae. Mulberry is found from temperate to subtropical regions and they can grow in a wide range of climatic, topographical and soil conditions. In most countries, including Turkey, Greece and Iran, mulberries are grown for fruit production rather than foliage (Ercisli, 2004; Mulberry fruits can be used as a worming agent, as a remedy for dysentery, and as a laxative, odontalgic, anathematic, expectorant, hypoglycaemic and emetic (Baytop, 1996). Phonemics possess a wide spectrum of biochemical activities such as antioxidant, ant mutagenic and anticarcinogenic properties, as well as the ability to modify gene expression (Nakamura, *et al.*, 2003; Mulberry fruit contains essential fatty acids that humans cannot synthesise, and must be obtained through diet. One of the most important points about mulberry fruits is its sensitive to environmental condition in harvesting time and the methods of harvesting that is not suitable. Because of this mentioned problem, mulberry fruits have a lot of waste. In animal feed industry, agricultural by-products were used in animal and poultry nutrition. One of the greatest challenges to a nutritionist is to formulate diets that adequately meet the nutrient requirements of the animal without having to provide excessive quantities of the expensive nutrients. In order to formulate the diets economically, it is essential that nutrient content of candidate ingredients analyzed as detail as possible. There was no information about mulberry fruits wastes nutritive value.

Materials and methods White fruits waste were sampling from 15 gardens reserved at Tehran province. Proximate analysis was conducted according to AOAC (1990) methods and mineral assay was conducted with atomic absorption. To determine metabolizable energy 8 mature Rhode Island Red (RIR) cockerels were used in Sibbald methods (1989). The cockerels were housed individually in metabolic cages in a temperature-controlled room with 12 h of light per day. Endogenous losses were obtained from 4 birds in 4 replicate. A group of 4 cockerels were used for mulberry fruits. The experiment protocol of McNab and Blair (1988) was used for the measurement of endogenous energy losses (EEL), AME and TME with the modification that 40g of each feedstuff were tub fed after 48 hours starvation rather than 50 g. All of the data were subjected to one- way analysis of variance test. Statistical significances among treatment means were determined by the method of new multiple range test of Duncan (1995) when the F value was significant at 0.05.

Results The result showed that mulberry fruits waste can be a energy source feedstuff, but its crude protein percentage is very low. NFE is a major part of mulberry fruits waste dry mater (81.1%). The result of mineral assay showed that mulberry fruits waste is a good source for iron and zinc but it had high potassium (0.68%) content. However high level of K may be makes some problem in poultry diets. The AME, AMEn, TME and TMEn content of white mulberry fruits waste were 12.99, 13.62, 17.14 and 16.64MJ/kg, respectively.

Table 1 The proximate analysis and minerals of white mulberry fruits waste (%)

DM	CP	CF	EE	Ash	NFE	
96.8	4.4	8.8	1.6	4.1	81.1	
N	Ca	P (%)	K	Mg	Fe (ppm)	Cu (ppm)
0.71	0.48	0.12	0.68	0.13	185	6.2

Table 2 Gross and metabolizable energy of white mulberry fruits waste(MJ/Kg).

GE	TMEn	TME	AMEn	AME
17.13	16.64	17.14	13.62	12.99

Conclusions According to this result, the metabolizable energy of mulberry fruit waste is similar to corn grain, but its crude protein content is half of corn grain. As the price of corn grain is 2.5-3 time of mulberry fruit waste cost, we can use this material as a source of energy in a low level for poultry nutrition. When the AME and TME systems were compared, TME and TMEn values for the mentioned feedstuff were higher than the corresponding AME and AMEn values. The Data from the present study provide new information on the ME values of white mulberry fruits waste and increase the possibility of using these in poultry nutrition.

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Protein fermentation characteristics in rumen fluid determined with the gas production technique

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Introduction The gas production technique was developed to determine the fermentation kinetics of organic matter in rumen fluid. However, the gas production technique can be adapted for the determination of protein fermentation characteristics. To do that the buffer must be N-free. All the N coming with the rumen fluid must be incorporated into microbial mass. This can be done by supplying the buffered rumen fluid with an excess of fast fermentable carbohydrates. To prevent a too high input of N from the rumen fluid the rumen fluid can be diluted further compared to the standard 3 times (Cone *et al.*, 1996). This makes N the limiting factor for fermentation and the obtained gas production profiles reflect the availability of N from the feed samples. The aim of the present study was to investigate if the adapted gas production technique is suitable to determine differences in protein availability in rumen fluid. The fermentation characteristics of N of 19 feed samples were determined using the adapted gas production technique. The amount of sample incubated, was that sufficient to provide 15 mg N. The results were compared with data of N degradation obtained with the nylon bag technique.

Materials and methods Gas production incubations were performed as described by Cone *et al.* (1996), with the exception that ammonium carbonate was replaced by NaHCO₃ (Cone *et al.*, (2005) and that the rumen fluid was diluted 20 times with buffer/mineral solution instead of the usual 3 times. The rumen fluid was obtained from 2 non lactating Holstein Friesian cows receiving 1 kg of concentrate in the morning and *ad libitum* grass silage. To bind all N coming with the rumen fluid, 3.33 g/l glucose, 3.33 g/l xylose and 3.33 g/l soluble starch was added to buffered rumen fluid and gas production was recorded. After 4 h of incubation gas production was ceased, indicating that no N was available for further fermentation. After 4 h an amount of sample providing 15 mg N for each of the 19 different samples was added to the bottles in duplicate, retaining an excess of quickly fermentable carbohydrates available. Under these conditions the availability of N from the samples determines the rate and extent of the gas production. The N degradation in the rumen for the 19 samples was also determined with the nylon bag technique. Samples were incubated in triplicate in three lactating Holstein Friesian cows each, for 0, 3, 8, 16, 48 and 336 h. The washout (W), undegradable (U) and degradable (D) fractions of N were determined as well as the rate of degradation (kd, 1/h) and the amount of rumen escape protein (REP) was calculated.

Results Figure 1 shows the gas production profiles determined by the N availability of some of the investigated samples. It is shown that there were large differences in both rate and extent of gas production. Comparing the gas production results with the nylon bag data showed that there was a moderate relationship between gas production after 5 h (GP5) and W ($r^2 = 0.63$) and between GP25 and kd ($r^2 = 0.52$). However, there was a rather good relationship between the gas production after 12, 15 and 20 h and the calculated amount of REP ($r^2 = 0.83$).

Conclusions The adapted gas production technique can be used as a rapid screening methodology to determine differences in N availability between samples and provides a good estimate of the amount of rumen escape protein.

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Figure 1 Gas production profiles of fast fermentable carbohydrates in an N-free medium, after a pre-incubation of 4 h to bind all available residues of N, supplemented with different amounts of samples, all containing exactly 15 mg N. The blank was not supplemented with N.

In vitro rumen degradability, ammonia levels and pH of different spices

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Introduction In developing countries, low quality forages (LQF), like cereal straws are the main basal feed for ruminants. But these forages are low in nutrient contents and so the ruminants cannot utilize these forages effectively which results in poor ruminant health and production. So it is needed to improve the quality of these forages. Conversely, rumen fermentation of these forages may result in considerable energy and protein losses as methane and ammonia N which cause pollution to the environment. So it is needed to find out additives that can also be used as supplements to reduce nutrient loss as methane and nitrogen. It would be desirable if these supplements or additives improve the efficiency of nutrient use in the rumen as well. Herbs and spices can be used as alternative sources. Researchers have been using plant extracts to manipulate rumen fermentation. But processing extracts from plant products will be costly and will require expensive instruments. Khan and Chaudhry (2008) have reported the potential in using spices to modify degradability and fermentation of different forages. However, it may be helpful to observe the degradability and fermentation profiles of these spices alone before their use as additives to improve forage degradability perhaps by stimulating the fibrolytic and or inhibiting the undesirable microbes. Therefore, this study compared the *in vitro* dry matter degradability (IVD) and rumen ammonia level (AL) and pH of different concentrations of selected spices at different times.

Materials and methods Two duplicated 5x3 factorial trials assessed the IVD of 5 spice (S) (Cinnamon=Ci, Clove=Cl, Coriander=Co, Cumin=Cu and Turmeric=Tu) containing crude protein (CP) 72, 102, 106, 223 and 86 (g/kg) respectively at 3 concentrations (C) (0.1, 0.2 and 0.4g) during *in vitro* incubations for 48 and 144h. The 144h incubation was selected to observe if the spices can continue to degrade during this longer time of incubation. Rumen fluid (RF) was obtained from 2 fistulated sheep. The RF was strained through a cheese cloth into insulated flasks under CO₂ before mixing with pre-warmed buffer at 1:4 ratio to prepare inoculum. Samples of 0.1, 0.2 and 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. Then the pH of the buffered rumen fluid was measured. The liquids and residues were separated by centrifuging the tubes at 3000 rpm for 10 minutes. The residues were washed with distilled water and dried at 60°C for 48h to determine IVD. The supernatant was collected, acidified with 1N HCl and analysed for AL by using a colorimeter at 660 nm. The data were analyzed by using General Linear Model of Minitab to study the main effects of S, C and their interaction (SxC) on IVD, AL and pH at each incubation time at (P<0.05).

Results Main effects of S, C and SxC were significant for IVD, AL and pH for each incubation time (P<0.05) (Table 1). The IVD was highest for Cu but lowest for Ci at each time. IVD reduced with the increase of C (P<0.001). IVD for all spices and AL and pH of buffered RF was greater at 144h than 48h. AL was higher in Cu and Co compared to other spices and increased at higher C but decreased for other spices at higher C. The pH increased with the increase of time and it was lowest in Tu compared to other spices at each incubation time.

Table 1 Mean IVD, AL and pH of different spices at different concentrations (SxC interactions) for 2 incubation hours (IH)

IH	C (g/40ml)	IVD (g/kg)					AL (mg/L)					pH				
		Ci	Cl	Co	Cu	Tu	Ci	Cl	Co	Cu	Tu	Ci	Cl	Co	Cu	Tu
48	0.1	325	746	701	764	637	40	55	129	146	36	7.37	7.18	7.30	7.38	7.19
	0.2	276	636	693	795	511	34	34	163	164	19	7.24	7.12	7.29	7.28	7.08
	0.4	259	528	688	745	416	4	20	183	173	11	7.13	7.02	7.15	7.18	6.97
Pooled SEM		33.2					12.4					0.023				
P for S, C and SxC		S=0.001; C=0.001; SxC=0.001					S=0.001; C=0.4; SxC=0.001					S=0.001; C=0.001; SxC=0.001				
144	0.1	409	804	719	841	867	88	96	164	186	94	7.71	7.60	7.70	7.68	7.47
	0.2	389	795	714	813	840	65	77	186	199	75	7.56	7.58	7.60	7.50	7.27
	0.4	353	637	704	789	643	36	35	198	204	9	7.43	7.35	7.54	7.40	7.12
Pooled SEM		30.9					12.4					0.033				
P for S, C and SxC		S=0.001; C=0.001; SxC=0.001					S=0.001; C=0.001; SxC=0.001					S=0.001; C=0.001; SxC=0.001				

Conclusions Higher CP in Cu and Co might have increased the availability of ammonia in the rumen and caused higher AL at higher C whereas lower CP in Ci, Cl and Tu caused lower AL at higher C. Cu and Co showing greater IVD and AL can be used to increase the utilisation of LQF by ruminants. Conversely, Tu and Cl, offer potential for their use with highly degradable animal feeds to reduce ammonia N losses during chewing and rumen fermentation. Further investigations are continued to determine the effects of these spices with different types of animal feeds on degradability and other fermentation profiles.

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Determining of nutritive values of tomato pomace using *in situ* and gas production techniques

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Introduction The amount of by-product feedstuffs generally increases as the human population increases and economies grow. Large quantities of by-products are used in the ruminant's diet in agro-industrial areas. (Gasa, *et al.* 1989). However, little is known about their fermentation pattern in the rumen and a better understanding of their digestion and products of fermentation is necessary in order to properly balance their introduction into the diets and the knowledge about their potential feeding value is insufficient.

Materials and methods The samples of tomato pomace were collected from tomato paste factory in northwestern of Iran. Tomato pomace was analyzed for DM and CP (AOAC, 2005), NDF and ADF (Van Soest *et al.* 1991). Three ruminally fistulated sheep (49±2.6 kg) used. The sheep were fed a diet consisting of 400 g kg⁻¹ concentrate and 600 g kg⁻¹ 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, 4, 8, 16, 12, 24, and 48 h. *In situ* DM and CP rapidly degradable fraction (a), potentially degradable fraction (b) and rate of degradation of fraction b (c), were calculated according model of Ørskov and McDonald (1979) as $y=a+b(1-e^{-ct})$. The mentioned animals were used as donors of ruminal fluid for the preparation of inoculums. Rumen fluid was collected 2 h after the morning feeding, filtered from cheese cloth, pooled and prepared a composite sample. Gas production was measured after 4, 8, 16, 12, 24 and 48 h of incubation in triplicate using a water displacement apparatus (Fedorak and Hrudehy, 1983).

Results The chemical composition of Tomato pomace was shown in Table 1. The degradability parameters of DM and CP and gas production data are shown in Table 2.

Table 1 The chemical composition of Tomato pomace

Feed	%DM	%CP	%NDF	%ADF	%ADIN
Tomato pomace	91.46	22.21	54.26	45.87	1.90

Table 2 *In situ* DM, CP disappearance (% of DM) and degradation characteristics and gas production (mL g⁻¹ DM)

	Incubation time (h)							DM and CP degradation characteristics		
	0	4	8	12	16	24	48	a ¹	b ²	c ³
<i>In situ</i> DM	6.3	18.6	25.8	29.8	35.4	42.0	48.6	7.09	43.06	0.068
<i>In situ</i> CP	10.0	16.3	28.2	30.3	32.9	42.5	56.1	10.38	54.43	0.037
Gas production	-	51.1	72.7	99.1	122.1	143.3	170.4	-	-	-

1: Rapidly degraded fraction (%); 2: Slowly degraded fraction (%); 3: Rate of degradation (fraction/h)

Conclusion Regarding to the limited available information on the degradability of this by-product and variation of their composition depending on the composition of original plant material, method of processing and type of components extracted or removed, the further researches are required for evaluation of this by-product. (Getachew, *et al.* 2004). Low gas yield for tomato pomace in initial incubation times was resulted due to high content of slowly fermented carbohydrates in tomato pomace. Tomato pomace had high level of NDF and it needs more time to attachment of micro organism. also resulting of low DM and CP ruminal disappearance

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Increasing nutritive value of white grape pomace by ensiling and drying for ruminants nutrition

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Introduction There is increasing demand for efficient use of animal food by products due to economic and environmental concerns. Agricultural by products such as tomato pomace, citrus pulp and grape pomace (GP) may be substituted advantageously to tackle animal feed shortage. Alipour and Rouzbehan (2006) demonstrated that GP tannins have adverse effects on nutrient utilization and other nutritional parameters in ruminants. Ensiling (Makkar, 2003) and drying (Martin-Garcia and Molina-Alcaide, 2007) can be used as a method of decreasing and inactivation of tannins. The aim of this study was to determine the effects of ensiling and drying on chemical composition, tannin content and improving nutritive value of GP.

Material and method Fresh grape pomace (FGP) was collected from commercial processor (TATAO fruit juice factory, Urmia, Iran). Silages were prepared using a small scale system of silage fermentation. Three samples of GP ensiled for 20 (EGP₂₀) and 45 (EGP₄₅) days in plastic vessels. Wet GP were oven dried at 65°C for 48h (DGP) that similar to ensiled treatments were made in three samples and proximately analysed according to AOAC (2003) (Table 1). Dry matter (DM) content was determined at 105 °C for 24 h in forced air oven. Also determinations of nitrogen were conducted using the micro-Kjeldahl method in an automated Gerhardt apparatus and crude protein (CP) was determined as nitrogen $\times$ 6.25. The NDF was assayed with sodium sulphite and without alpha amylase. Both NDF and ADF were expressed without residual ash. Acid detergent insoluble nitrogen (ADIN) was determined by Kjeldahl analysis of ADF residues. Total phenolics (TP) were measured using the Folin Ciocalteu method (Makkar, 2000). Total tannin (TT) was determined after adding insoluble polyvinylpyrrolidone (PVPP) and reacting with Folin Ciocalteu reagent. Tannic acid was used as the standard to express the amount of TP and TT. Data were subjected to ANOVA statistical analysis and mean values were compared with Duncan test.

Results The chemical composition of GP samples (FGP, DGP, EGP₂₀ and EGP₄₅) are given in Table 1. DM content of EGP decreased compared to FGP. The losses during the preservation mainly based in the oxidation of soluble carbohydrates. Consequently, the water generated in the oxidations led to a significant decrease of DM content. Crude protein contents of GP ranged from 12.9 to 14.4%. Ensiling and drying had no effect on OM and CP concentrations. Investigation on GP showed that ensiling and drying increased acid detergent insoluble nitrogen (ADIN) values. The GP initial pH (FGP) was 3.89, but at the end of ensilage process, The EGP₂₀ and EGP₄₅ pH reached to 3.74 and 3.68, respectively. Moreover, crude fiber (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin (ADL) values increased in experiments significantly ($P < 0.01$). Drying and ensiling inclined on total tannins (TT) and total phenolics (TP) significantly ($P < 0.01$). That this gradual reduction could be due to the polymerization or oxidation of tannins in GP.

Table1 Mean chemical composition, total phenolics, total tannins (g/kg DM) and pH of fresh, dried and ensiled GP

Item	Treatments				P-value	S.E.M
	FGP	DGP	EGP ₂₀	EGP ₄₅		
DM	381 ^c	940 ^d	285 ^b	225 ^a	**	14.75
OM	968 ^d	928 ^a	955 ^c	944 ^b	**	28.5
CP	139 ^b	132 ^a	129 ^a	144 ^c	**	5.5
CF	190 ^a	193 ^a	250 ^b	271 ^c	**	4.5
NDF	496 ^a	504 ^b	552 ^c	593 ^d	**	3.75
ADF	381 ^a	389 ^b	473 ^c	571 ^d	**	4.5
Lignin(sa)	201 ^a	221 ^b	285 ^c	381 ^d	**	8.25
ADIN	0.089 ^a	0.094 ^b	0.1 ^c	0.13 ^d	**	3.9
pH	3.89 ^c	3.92 ^c	3.74 ^b	3.68 ^a	**	0.00
TT	20.5 ^d	18.6 ^c	15.5 ^b	9.2 ^a	**	3.75
TP	24.8 ^d	23.6 ^c	18.7 ^b	13.2 ^a	**	1.75

Conclusion Ensiling and drying reduced the levels of tannins and phenolic compounds of GP. Thus, they may be considered as appropriate ways to improve nutritive value of GP. Furthermore, appears that ensiling time be efficient on diminution of phenolics composition and increasing nutritive value of GP. More investigation should be done on different varieties of GP to detannification and inactivation of tannins and phenolics composition.

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The influence of thyme and pennyroyal essential oils, found to be effective as acaricides against the poultry red mite (*Dermanyssus gallinae*), on occurrence of taint in hens' eggs

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Introduction Conventional pest management (the use of synthetic pesticides) is being increasingly hampered by issues of pest resistance, tighter legislation and greater consumer demand for food stuffs produced in an environmentally friendly manner. A growing body of research suggests that essential oils may be of use as an alternative to synthetic products in managing the poultry red mite (*Dermanyssus gallinae*), a serious pest of egg production systems for laying hens (Kim *et al.*, 2004). It is necessary to ensure that any essential oil recommended as an acaricide against this pest does not taint the eggs produced from hens in poultry units in which the oil would be deployed. The aim of the current study was to test the hypotheses that thyme and pennyroyal essential oils, having been found to be effective as acaricides for *D. gallinae* in preceding work, would have no effect on the occurrence of taint in hens' eggs.

Materials and methods A panel of six consumers was asked to evaluate the odour of eggs on a sliding scale (where a higher score indicated a stronger smell) according to a pre-prepared questionnaire. Consumers were unaware of the treatments from which the experimental eggs originated and so carried out the sniff testing blind to avoid bias. Consumers were asked to score responses for the following five attributes: strength of abnormal smell, pleasantness/foulness of this smell, and positive/negative effect of the smell on attitude towards buying a particular egg. Eggs were either commercial in origin or had been collected (on three separate occasions) from experimental poultry huts (containing Lohmann Brown pullets) that had been subjected to one of three treatments (on a weekly basis): un-treated (no solvent), treated with solvent only (water), or treated with either thyme or pennyroyal essential oil at a concentration (mg/cm³) 5x that previously determined to kill 90% of *D. gallinae* adults. Eggs were present in huts during product application and removed 24 hours after. Gas chromatography mass spectrometry (GCMS) was also conducted on these eggs to investigate taint from the essential oils, having first shown that various compounds from the essential oils could be extracted (in hexane) from combined egg yolk and albumen and used as identifiers of taint after eggs were artificially spiked with either oil.

Combined data from all sampling occasions were subjected to a two-way analysis of variance (ANOVA) carried out on the data from the questionnaire, with person and score considered as factors for the results from each question. Tukey tests were run where ANOVA indicated a significant treatment effect. For results on the foulness of smell, data were transformed before analysis (arcsin square-root) to improve the fit of the residuals from the ANOVA to a Normal distribution (where all residuals were checked for Normality using the Anderson-Darling Test). Statistics were undertaken in Minitab (version 14).

Results GCMS analysis of egg samples spiked with essential oil showed that certain compounds from the oils could be easily and reliably identified in eggs at a concentration as low as one part oil in 8 000 parts egg. For eggs spiked with pennyroyal essential oil, only pulegone was reliably identified by the GCMS protocol used. For eggs spiked with thyme essential oil, moslene, benzene, linalool and thymol were identified by GCMS. These compounds were subsequently adopted as identifiers for any taint that might occur in eggs taken from experimental poultry huts.

Results of GCMS did not identify any essential oil compounds as being present in eggs laid by hens under different essential oil treatments.

Despite finding no egg taint using GCMS analysis, differences were detected between treatments by the consumer sniff-test panel for a number of responses to eggs. ANOVA found a significant difference ($P < 0.05$) between treatments for strength of abnormal smell, foulness of smell and for a negative effect of smell on attitude towards buying an egg. Differences between pairs of means (treatments), as identified by Tukey's Test ($P < 0.05$), demonstrated that in all cases eggs exposed to pennyroyal essential oil scored significantly higher (i.e. less favourably) than other treatments.

Conclusions The results suggest that it should be possible to deploy thyme essential oil for *D. gallinae* management with no risk of egg taint influencing consumer attitude towards eggs and egg purchasing. Furthermore, the current use of thyme (and/or its main compound thymol) in pest management where food stuffs such as honey are concerned (Donders *et al.*, 2006) might add further weight to the acceptance of this product for use in poultry pest management by both the egg industry and consumers. The use of pennyroyal essential oil resulted in egg taint detectable to consumers, suggesting that this oil would not be suitable for use as a *D. gallinae* acaricide where laying hens are present in the poultry house.

The GCMS analysis conducted could not provide support for the egg taint detected by the consumer sniff-test panel. More sensitive analytical methods or extraction techniques may be required to confirm or deny that compounds such as pulegone (the main component of pennyroyal essential oil) were responsible for pennyroyal essential oil tainting hens' eggs.

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A genetic-epidemiological model for Marek's Disease in Poultry

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Introduction Marek's disease (MD), caused by a herpes virus, is a very infectious, lymphoproliferative and chronic disease of poultry. Breeding for improved MD resistance poultry stock is possible since MD resistance has been associated with MHC haplotypes, QTL and candidate genes. However, integration of host genetics vis-à-vis other control strategies and the utilisation of genes or gene markers for MD in practical breeding programmes is still a challenge. The objective of the present study was to develop a basic genetic-epidemiological model for Marek's disease infection in poultry, identify parameter spaces that describe the disease dynamics correctly and investigate the impacts of possible genetic and vaccination control strategies on overall disease dynamics.

Materials and methods The transmission of infection by MDV may be described by compartmental models (Anderson and May, 1992), in which individuals move from one state to another. A compartmental model considering different infective phases of MD within the host system, as described by Witter and Schat (2003), was considered. These are susceptible (S), exposed (E), cytolytic phase 1 (C_1), latent (L), cytolytic phase 2 (C_2) and proliferative (P). The transmission coefficient (β) denotes the expected number of new infections per infectious animal per susceptible animal per day. The infectivity (α) of the virus depends on virulence and shedding rates by individuals in different phases. The recovery rate (γ) is the expected number of recoveries per infected animal per day. The mortality rate (μ_d) implies disease-dependent mortality, which is the expected proportion of individuals dying per day among the infected individuals. Additionally, the model also considers disease-independent mortality (μ_i), which is the expected proportion of individuals dying per day due to other causes. The model was simulated stochastically as described by Nath *et al.* (2008). A homogenous closed population of 10,000 birds was assumed and simulated for 500 days. Possible durations of stay in compartments E , C_1 , L and C_2 were specified as 7, 7, 21 and 21 days, respectively (Witter and Schat, 2003). The contact rate between individuals was 1.0 and disease-independent mortality was 0.0002 per day. Different ranges of α , γ and μ_d at E , C_1 , L , C_2 and P were explored to identify parameter values that mimicked the MD epidemiology pattern. The model was explored further using parameters thought plausible. Stochastic simulation of 1000 replicates was carried out in each case. If more than 10% of individuals in the population died, the epidemic was deemed as being major.

Results The model indicated that $\beta = 0.00005$ and $\gamma (C_1) = 0.1$ resulted in a basic reproductive ratio (R_0) of 5.80 with a probability of a major epidemic greater than 0.84. These outcomes closely reflect scenarios for unvaccinated poultry flocks (Witter and Schat, 2003). In case of $\beta = 0.00005$ and $\gamma (C_1) = 0.9$, the resulting R_0 value was 0.59 and the proportion of a major epidemic was 0.19. The proportion of exposed individuals was high (0.98), but there would not be any visible clinical sign of MD among the birds in E phase. Most of the exposed individuals would be immunologically able to remove the virus (0.84) and only a small proportion (<10%) would die due to MD (Table 1). These characteristics of MD infections are akin to a population vaccinated with MD vaccine (Witter and Schat, 2003). An increased interval for the phase L to C_2 decreased MD-related mortality, while a decreased transmission coefficient decreased the major epidemic probability. The model also identified that whilst the proportion of infected individuals in a genetically resistant population may be high, the incidence of disease will still be rare, since infection will rarely cause obvious clinical cases of disease.

Table 1 Average proportion of different events (infection, recovery and mortality) at various phases of MD infection

Vaccination status	Event	Phases of infection				
		E	C_1	L	C_2	P
Unvaccinated	Infection	0.99	0.91	0.53	0.50	0.41
	Recovery	0.06	0.37	0.01	0.00	0.00
	Mortality	0.00	0.00	0.01	0.08	0.40
Vaccinated	Infection	0.98	0.90	0.12	0.11	0.09
	Recovery	0.06	0.78	0.00	0.00	0.00
	Mortality	0.00	0.00	0.00	0.01	0.08

Conclusions The proposed genetic-epidemiological model captured the epidemiology and underlying immunogenetic mechanisms of MD for vaccinated and unvaccinated cases. With appropriate alterations of parameters, the present model of MD could be used for evaluation of different genetic and non-genetic strategies to control MD in poultry populations.

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The effects of *in ovo* feeding of threonine and carbohydrates on growth performance of broiler chickens

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Introduction The few days before and after hatch is a critical period for the development and survival of commercial broilers. Glycogen reserves in the chick embryo are significantly depleted during the perihatch period in order to meet the high energy demands during the process of emergence (Uni and Ferket, 2004). The immediate posthatch period is characterized by a transition from the use of lipid-rich yolk as the nutrient source to exogenous feed rich in carbohydrates and proteins. This transition is accompanied by rapid physical and functional development of the gastrointestinal tract (Sklan, 2001). The accelerated rate of development posthatch is reflected in the several-fold improvement in the development of the gastrointestinal tract and organ growth which will allow for a more efficient uptake of nutrients for muscle development. During late embryogenesis, solutions administered into the amniotic fluid (*in ovo* feeding) are consumed by the embryo, digested, and absorbed by the embryonic intestine prior to pipping (Uni *et al.*, 2005). *In ovo* feeding of supplemental nutrients may help to overcome the constraint of limited egg nutrients (Foye *et al.*, 2006). The objective of this experiment was to evaluate the effects of *in ovo* feeding of carbohydrates and threonine, alone or in combination, on growth performance of broiler chicks from 1 to 42 d of age.

Materials and methods. At 16 d of incubation, 480 fertile eggs were weighed and evenly distributed into 4 treatments with 4 replicates per treatment and 30 eggs per replicate. On day 17.5 of incubation, 1 ml of *in ovo* fed solutions were injected into the amniotic fluid of eggs. The solutions were: I) Carbohydrate (CHO) solution: 25 g of maltose/L, 25 g of sucrose/L, 200 g of dextrin/L in 0.5% saline, II) Threonine (THR) solution: 1% threonine in 0.5% saline and III) THR+ CHO solution in 0.5% saline. The controls were not injected but they were subjected to the same handling procedures as the *in ovo* treatment groups. Percentage hatchability was calculated and body weight of hatched chicks, were recorded after hatch and then chicks were transferred to a research farm. Each treatment group of chicks was randomly assigned to 4 floor pens. All birds had *ad libitum* access to water and feed. Diets were corn-soybean meal based formulated for starter, grower and finisher to meet or exceed the NRC requirements and recommendations of the primary breeder, identical in all experimental groups. Data were analyzed using the GLM procedure of SAS (2001). Mean separation was accomplished using Duncan's multiple range tests.

Results Hatchability of fertile eggs did not differ significantly among treatments although administration of CHO and THR+ CHO solutions resulted in about 4% increase in hatchability. Hatching body weight of chicks and chick BW: initial egg weight ratio of *in ovo* fed hatchlings was higher than in controls ($P < 0.01$). Chicks from eggs injected with 1% THR and 1% THR + CHO had significantly greater body weight gain at 42 d compared with the chicks of eggs injected with CHO and also controls. Chicks from eggs injected with 1% THR had the lowest feed conversion ratio among treatments (Table 1).

Table 1 The effects of *in ovo* feeding of Threonine (THR), Carbohydrates (CHO) and THR + CHO on hatchability, hatching body weight (BW), 1-42 d weight gain and feed conversion ratio (FCR) of broilers.

Treatments	Hatchability (%)	BW of hatched chicks (g)	Hatched chick BW/ Initial egg weight (%)	Weight gain 1-42 days (g/bird)	FCR 1-42 days
Control	86.6	41.2 ^b	78.6 ^b	2006.1 ^b	1.96 ^a
THR	87.7	42.2 ^b	80.4 ^a	2138.8 ^a	1.79 ^b
CHO	91.1	42.4 ^b	80.8 ^a	2091.1 ^{ab}	1.85 ^{ab}
THR + CHO	91.1	43.1 ^a	82.1 ^a	2131.4 ^a	1.88 ^{ab}
SEM	3.05	0.27	0.52	29.8	0.035
Significance	n. s.	**	**	*	*

Conclusions Our results showed that *in ovo* feeding of THR and THR + CHO at 17.5 d of incubation can improve hatchling weight, body weight gain and FCR of broilers post-hatch. The injected nutrients may provide the fuel for hatch and subsequent development and growth.

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Intensive selection for growth in broilers has not altered normal feeding behaviour

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Introduction Selection for increased growth rate in livestock may be accompanied by increases in requirements for energy and nutrients. It has been suggested that intensively selected broilers have altered food intake control mechanisms and could be constantly hungry, due to their high resource demands (Bokkers *et al.* 2004), which would be a major welfare issue. Such alterations in food intake control mechanisms as a side-effect of genetic selection would lead to changes in the feeding behaviour of birds, such as the clustering of visits into meals and the probability of birds starting a new meal in relation to the time since the last meal. The aim of this study was to test whether broilers intensively selected for growth showed any alteration in the structure of their feeding behaviour that would indicate a change in the underlying hunger and satiety control mechanisms.

Materials and methods Data of visits to feeders from 16,823 broilers aged between 2 and 5 weeks from four genetic lines in 12 hatches per line were used for analysis. Lines differed in their degree of selection for growth, with average live weights of 2.4, 2.1, 1.8 and 1.6 kg at 35 days of age for lines A, B, C and D, respectively, with all birds being fed on the same high quality food. Visit data was grouped into meals using meal criteria, were estimated per hatch within lines as described by Howie *et al.* (2008). The probability of birds starting a meal within the next min (Pstart) was calculated as the number of intervals $> t$ and $\leq t + 1$ divided by the number of intervals $> t$, where t = interval length in minutes between two subsequent visits to feeders by the same bird. Differences in bouting between the lines were analysed by calculating the frequency ratios between the relative frequencies of the bin containing the meal criterion and the bin containing the peak in frequency of the long intervals, using histograms of intervals between visits. Meal characteristics were compared between lines using ANOVA in GENSTAT 11, and the effect of stocking density was estimated using REML analysis, with stocking density within line as a fixed effect.

Results No significant difference in meal criteria was found between the lines. Similar patterns of bouting were observed across all the lines, and even the line with the highest growth rate showed a normal bouted structure (Figure 1). The faster growing lines (A and B) took longer and larger meals, but fewer per day (all $p < 0.001$), than the slower growing lines (C and D), even when the effects of differences in stocking density were accounted for. Birds of all lines showed a low probability of starting to feed immediately after a meal and similar proportional increases in this probability with time for lines A, C and D but slightly lower values for line B. As a result of the large number of data included in the analyses, small differences between the lines in the degree of bouting and change in starting probability were statistically significant but did not vary consistently with selection intensity.

Figure 1 Histogram of intervals between visits for line A. The proportion of intervals in the first bin (0.52) is not shown for clarity of the distribution at longer intervals; the same pattern was observed in all lines.

Conclusions No alteration of the overall organisation of short-term feeding behaviour into bouts in relation to selection intensity was found. This study suggests that although there are differences in meal patterning between genetic lines in these broilers, the structure of short-term feeding behaviour that results from intensive genetic selection for growth is similar to that observed in many other species, ranging from insects to horses. The similar proportional increase in the probability of birds starting a new meal with time since the last meal and the lower meal frequency of lines A and B compared to C and D is not consistent with the hypothesis that intensive selection for growth leads to birds that are constantly hungry.

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What contributes to differences in phenotypic variation between generations?

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Introduction Under the animal model genetic variance is estimated in the base population taking into account inbreeding and is otherwise assumed to remain unchanged over generations. In practice, phenotypic variation differs randomly or systematically over time. Intuitively, such changes would be attributed mostly to environmental effects, and so lower heritability would be expected when variation is inflated. Studies in dairy cattle show contradictory results (e.g. Boldman and Freeman, 1990). Laying hens are kept under environmental conditions intended to be constant, but show substantial heterogeneity in phenotypic variance (V_P) over generations. The aim was to investigate how variance components change.

Materials and methods Body weight was recorded on 88847 individuals from 7 fully pedigreed closed lines over 6 generations (Table 1). Lines A22, A33 and A88 are Rhode Island White and were selected on egg number and shell color and K22, K44, K66 (Rhode Island Red) and P11 (Barred Plymouth Rock) were selected on these traits and also egg weight. All birds were kept on the same farm of the Poultry Research Branch at Zakrzewo (Poland).

Table 1 Means (g) and standard deviations of body weight at 35 weeks of age over 6 generations

gen	A22		A33		A88		K22		K44		K66		P11	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
1	1646	198	1786	201	1633	180	1810	209	1806	158	1819	204	1357	185
2	2026	188	1812	204	2001	213	1844	174	1998	188	2124	207	1896	209
3	2072	182	1912	239	2012	227	2076	197	2084	177	2405	249	2001	234
4	1941	179	1811	212	1916	205	1964	179	1954	177	2102	198	1645	203
5	1926	168	1785	191	1861	187	1999	184	2008	178	2070	200	1987	189
6	1873	180	1702	191	1796	189	1903	176	1916	170	2066	196	1995	210

The following univariate animal model was used to analyze the data using ASREML (Gilmour *et al.*, 2006): $y = X\beta + Za + e$ where: y , β , a and e are vectors of, respectively, observations, fixed effects for sex and hatch week within generation, random additive genetic effects, and residual. X and Z are known design matrices. In the full model environmental variances (V_E) and genetic variances (V_A) were allowed to vary among generations, uncorrelated for V_E but with correlation 1 for V_A . Alternative models were considered by constraining one variance component or their ratio as fixed. Models were compared by the Akaike Information Criterion (AIC) incorporating differences in log likelihoods and number of parameters fitted.

Results In all lines the worst fit was observed for the model with fixed variance components, in most cases followed by that where only V_E was allowed to change (Figure 1). In three lines a model which allowed for changes only in V_A was close in fit to the full model. Under the full model a high proportion of changes in V_P was attributed to changes in V_A (Figure 2). The results indicate that a model assuming homogenous variances may not be tenable in some populations.

Figure 1 AIC values for the alternative models

Figure 2 Variance components under the full model

Conclusion Both genetic and environmental factors contribute to differences in phenotypic variance between generations. The causative factors influencing both the total and the components of variance are not understood.

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Liquid feed fermented with *Lactobacillus salivarius* reduces susceptibility of broiler chickens to *Salmonella enterica typhimurium* Sal 1344 nal^r

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Introduction Consumption of poultry meat is associated with human *Salmonella* infections (Revolledo *et al.* 2006). One way to control the presence of these bacteria in broiler flocks is to make chickens less susceptible to colonisation. Fermented liquid feed may be a potential tool to reduce the *Salmonella* carriage in broiler chickens (Heres *et al.* 2003). Fermented liquid feed is a moistened feed with high numbers of lactobacilli ($>10^9$ cfu/gr), high concentration of lactic acid (>150 mM) and a low pH (<4.5) (Brooks *et al.* 2003). In the present experiment, a strain identified as *Lb. salivarius* that has been isolated from chicken gut and has been selected for its probiotic and fermentation properties after a screening method, was assessed for its efficacy in reducing the shedding of *Salmonella typhimurium* in poultry.

Materials and methods Newly hatched chicks were obtained from a specific pathogen free (SPF) White Leghorn flock (SPAFAS) and housed in negative pressure isolators. A total of 68 hatchlings were randomly divided into four groups each of 17 birds. One group was provided with a daily dose of 10^7 cfu/ml of *Lb. salivarius*, delivered via drinking water from day one of age. The second group was provided with 10^9 cfu/gr of *Lb. salivarius* delivered in fermented liquid feed (FLF). The third group was provided with feed acidified with 30.3 ml of lactic acid/ kg of wet feed from day one of age and the last group was the control without any addition of *Lb. salivarius* or acid FLF was prepared by mixing commercial unmedicated pelleted feed with water at a ratio of 1 feed: 1.2 water, inoculating with 10^6 cfu of *Lb. salivarius* and incubating at 30°C for 24h. The acidified feed was formulated to contain the same amount of lactic acid as measured in the FLF; feed and water were also mixed with 1:1.2 ratio and prepared shortly before they were provided to broilers. Both feed and water were provided *ad libitum*. All groups were challenged by oral gavage with 10^6 cfu of a nalidixic acid resistant mutant of *Salmonella enterica typhimurium* Sal 1344 nal^r at two weeks of age. Cloacal swabs were taken from 10 tagged birds per group at least 2 times per week for 6 weeks prior to and after challenge to determine the total lactobacilli counts and the shedding of *Salmonella*.

Results The mean lactobacilli counts of the fermented feed treatment at the end of the experiment were significantly higher ($P<0.05$) than the three other groups (Figure 1). The percentage of the days which the 10 birds per group were free of *Salmonella* was 51% for the group fed fermented feed and was significantly ($P<0.05$) higher than the group of the acidified feed, which had 23% of *Salmonella* negative days, the group provided *Lb. salivarius* via water, with 19% of *Salmonella* negative days and the control which had 8% of *Salmonella* negative days. The acidified feed group showed significantly more negative days ($P<0.05$) than the control group.

Figure 1 The mean lactobacilli counts for each group Control (X), Water (♦), Acidified Feed (▲), Fermented Liquid Feed (■).

Conclusion The proportion of *S. typhimurium* – shedding chickens was decreased significantly in both chickens fed fermented liquid feed and acidified feed; though, the effect of acidified feed in the control of *Salmonella* was more limited than that of the fermented liquid feed. Also, as a means of controlling *S. typhimurium* infection providing lactic acid bacteria to poultry through fermented feed could be better than through water. It appears that there is a synergistic effect of both the high numbers of lactobacilli and their production of lactic acid, which reduces the pH in the gastrointestinal tract of the chicken, on the control of *Salmonella* status of chickens.

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The effect of feeding stearidonic acid enriched soya oil to broilers on the fatty acid composition and sensory characteristics of chicken meat

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Introduction Enriching chicken meat with long chain n-3 polyunsaturated fatty acids (LC n-3 PUFA) is a viable means of increasing population intakes (minimum recommended 450 mg/d, SACN, 2004) of these essential fatty acids. Feeding broilers fish oil to achieve this causes problems with taint in the meat, whereas feeding an oil rich in C18:3 n-3 (LNA) does not result in the deposition of LC n-3 PUFA (Rymer and Givens, 2006). Stearidonic acid (C18:4n-3) is further down the conversion pathway of LNA to LC n-3 PUFA and appears to be converted much more efficiently by humans to LC n-3 PUFA than LNA (James *et al.*, 2003). Feeding birds an oil rich in C18:4n-3 may therefore result in more enrichment of poultry meat with n-3 PUFA other than LNA, without the taint associated with feeding fish oil. The objective of this experiment was to determine what the effects on meat fatty acid composition and sensory characteristics were when broilers were fed an oil (SDA) derived from soyabean genetically modified to produce high concentrations of C18:4 n-3.

Methods Male, day old Ross 308 chicks (120) were reared for 14 d in a single group on a common starter diet containing conventional soyabean oil (CON). At 15 d, the birds were weighed and randomly allocated to one of 24 pens (five birds per pen). Pens were blocked in groups of three, and within each block, pens were randomly allocated to one of three treatment groups (eight replicate pens per treatment). Birds were fed identical grower (days 15-28) and finisher (29-50 d) diets supplemented with either CON oil, fish oil (FISH) or SDA. Diets were based on wheat, soyabean meal, sunflower seed meal and maize gluten meal; crude protein contents were 261, 275 and 230 g/kg and AME contents 12.2, 13.2 and 13.1 MJ/kg in the starter, grower and finisher phases respectively. Birds were weighed at 41d and sacrificed in blocks between 41 and 50d. Samples of skinless breast and leg meat were taken from all birds, composited by pen for analysis of fatty acid composition and composited by treatment for sensory analysis (quantitative descriptive analysis with ten assessors scoring meat between 0 and 100 for a range of descriptors), using either freshly cooked meat (breast and leg meat) or reheated meat (leg meat only). The effect of broiler diet on the meat's fatty acid composition and sensory characteristics were determined by analysis of variance.

Results There was no significant effect of treatment on bird performance between 15 and 41 d. FISH reduced LNA and increased LC n-3 PUFA content of meat (Table 1). SDA increased LC n-3 PUFA content compared with CON and total n-3 content (after deduction of LNA, to give an estimate of potential LC n-3 PUFA supply) was considerably higher with both FISH and SDA compared with CON. Broiler diet had no significant effect on the meat's appearance, or on the aroma or flavour of breast meat. However, fishy aromas, flavours and aftertastes were detected in the leg meat of FISH fed birds, particularly when the meat was reheated (Table 2). Significant fishy notes were also detected in the reheated leg meat of birds fed SDA.

Table 1 Effect of broiler diet on the meat n-3 fatty acid composition (mg/100 g tissue)

Fatty acid	Skinless breast meat			SEM	Sig	Skinless leg meat			SEM	Sig
	SDA	CON	FISH			SDA	CON	FISH		
LNA	116	80	31	9.8	**	231 ^a	175 ^a	89 ^b	21.2	*
LC n-3 PUFA	87 ^b	31 ^a	209 ^c	15.0	*	141 ^b	29 ^a	430 ^c	29.9	*
Tot. n-3 less LNA	318 ^b	34 ^a	222 ^b	26.1	*	583 ^b	39 ^a	466 ^b	47.3	*

*: P<0.05, **: P<0.01. ^{a,b} Mean values within a row without a common superscript differ significantly (P<0.05)

Table 2 Effect of broiler diet on the sensory scores (0-100) of leg meat

Fishy attribute	Freshly cooked meat			Reheated meat			LSD
	SDA	CON	FISH	SDA	CON	FISH	
Aroma	18.3 ^{ab}	12.7 ^a	29.1 ^c	23.6 ^{bc}	9.1 ^a	51.5 ^d	10.7
Flavour	20.1 ^{abc}	11.8 ^{ab}	23.0 ^{bc}	29.0 ^a	10.7 ^c	51.3 ^d	11.2
Aftertaste	14.5 ^{abc}	12.1 ^{ab}	22.5 ^c	18.8 ^{bc}	9.2 ^a	45.9 ^d	9.4

^{a,b,c,d} Mean values within a row without a common superscript differ significantly (P<0.0001)

Conclusions Feeding broilers SDA produced meat with nutritionally significant concentrations of LC n-3 PUFA or its immediate precursors (recommended intake of LC n-3 PUFA 450 mg/d, SACN, 2004) while reducing the taint detected when birds were fed fish oil.

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Bayesian estimation of posterior means of heritability for weight at week 8 in Iranian indigenous chickens using an animal model

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Introduction Indigenous poultry breeds are of a major importance to supply the rural population with eggs and meat in many developing countries of the world (Esmaelkhanian *et al.*, 2004). A successful animal breeding plan is a continuous process of elimination and searching in a way that breeder could be able to manipulate genetic variation for changing a population to optimise desired phenotypes (Williams *et al.*, 2002). As highlighted by Chambers (1990) animal's body weight, which could be easily measured, is probably the most frequently used indicator of growth at a specific age. In practical animal breeding programmes, the breeding objective is usually genetic improvement of production and reproduction traits for a population under consideration. Knowledge of genetic parameters is of crucial importance to be implemented for predicting breeding value of candidate animals. The main objective of this study was to estimate heritability of body weight at week 8 for Iranian indigenous chickens through Bayesian statistical approach using an animal model.

Materials and methods In this study a total of 6440 weight records at week 8 belonging to Iranian indigenous chickens were used to obtain Bayesian estimation of posterior means of heritability. All records were collected in a centre of indigenous chicken breeding located in Khorasan province, Mashhad. In the data file, the average weight at week 8 was 571.46 g. An animal linear model was applied for genetic analysis. In the model, the fixed environmental effects of sex and hatch number (4 hatches) were included. Furthermore, the weight of chicks (w) at day one (average 35.03 g) was fitted in the model as linear covariate. Random additive genetic effect of chicks (animal) was also put in the model. All data were from the first of generation. The number of chicks with records, sires and dams were 6440, 81 and 747 respectively. A Bayesian estimation approach was used to obtain posterior means of additive genetic as well as environmental variance components for weight at week 8. The variance components were estimated implementing MTGSAM software (Van Tassell and Van Vleck, 1995) through carrying out 100,000 Gibbs sampling in which the first 5,000 rounds were assumed as burning in period. The convergence criterion was set to be 0.0001. The statistical linear model was as follows:

$$y_{ijk} = \mu + sex_i + hatch_j + b * w_{ijk} + animal_k + e_{ijk}$$

Results Estimated additive genetic and environmental variance components were presented in Table 1. The posterior means of additive genetic ($\sigma_A^2 = 2331$) and environmental ($\sigma_E^2 = 3565$) variance components were 2331 and 3565 respectively. Posterior means of heritability of weight at week 8 was found to be approximately 0.4 which is in the range of body weight heritability estimates reported by (Chambers, 1990).

Table 1 Bayesian estimates of additive genetic, environmental and phenotypic variance components for weight at 56 d

Trait	No. Records	Variance components (g ²)			Heritability
Weight at 56 d	6440	$\sigma_A^2 = 2331$	$\sigma_E^2 = 3565$	$\sigma_P^2 = 5896$	0.4

Conclusion Heritability is an important elemental key to make genetic progress in a population. As the heritability of a trait increases the magnitude of selection response increases over a generation. The relatively high estimate of heritability obtained for weight at 56 d for Iranian indigenous chickens indicates that there is a great genetic potential for the population to be improved based upon genetically selecting individuals with high weight performance. However, as many traits are simultaneously considered in poultry breeding, multi traits animal models are needed to be used for obtaining an accurate estimate of genetic parameters.

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Effects of chronic progesterone injection on performance, plasma hormones and ovarian morphology of feed-satiated and fed restricted broiler breeder hens

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Introduction Single injection of chronic dose of P_4 in laying hens during the preovulatory open period has been shown to have positive effects on inducing a preovulatory LH surge and ovulation (Johnson *et al.*, 1985). However, chronic injection of P_4 has been shown to increase baseline concentrations of P_4 and result in arrested laying and disrupted distribution of hierarchical follicles in turkeys (Bacon and Liu, 2004). The current study was designed to examine the effects of a simulated progesterone surge (by injection of chronic dose of P_4) on broiler breeder hens performance, ovary morphology, plasma metabolites and hormones concentrations in either feed-satiated or feed-restricted hens.

Materials and methods A total of 64 Cobb 500 hens were fed either restricted or *ad libitum* from 27 to 38 wk of age. Fourteen laying hens from each group were selected to conduct P_4 injection assay. Half of the birds in each group ($n=7$) were injected daily with 2.5 mg P_4 /kg BW and the remaining birds were used as control. The P_4 injections were given subcutaneously, at the base of the neck, daily (at 0900 h) for 21 d. Settable and abnormal eggs were recorded daily. Blood sample were taken just before initiation of injections, 10-d and 20-d after initiation of injection. Plasma samples were analyzed for glucose, cholesterol, triacylglycerol (TAG), P_4 , estradiol (E_2), testosterone, T_3 and T_4 concentration. Ovulation rate was calculated as (normal eggs + soft shell eggs + 2×double yolk eggs). The experimental birds were housed in individual cages. The experimental design was completely randomized with factorial arrangement using individual broiler breeder hens as experimental unit. Performance data as well as plasma metabolites and hormones data were analyzed as repeated measures using Proc Mixed of SAS software (SAS Institute, Cary, NC).

Results and discussion Although egg production declined following P_4 injection (Fig.1), ovulation rate remained at high levels in injected birds in the first week after initiation of P_4 injection (Fig 2). Progesterone injection in feed-satiated and feed-restricted birds resulted in ovary regression; the ovary of these birds having no hierarchical follicles. Progesterone injection increased incidence of holding hard-shelled eggs in the uterus. Progesterone injection depressed plasma E_2 concentration (Fig 3) in both *ad libitum* and restricted fed hens. Hens with free access to feed had significantly lower plasma E_2 levels compared to restricted fed hens. Our results revealed that whereas injecting chronic dose of P_4 induced frequent ovulation early in the injection period in both feed- satiated and feed-restricted breeder hens; however this higher ovulation rate did not result in more settable egg production. Restricted fed and *ad libitum* fed laying breeder hens respond in a similar way to chronic dose of P_4 injection.

Figure 1 Settable egg production (%) of hens

Figure 2 Ovulation rate (%) of hens

Figure 3 Plasma estradiol concentration of hens from different treatments

A: *ad libitum*, R: Restricted, I: Injected, N: Non-injected.
Data are means $\pm$ SEM. a-c Data points with different letter are significantly different at the age of the hens ($P<0.05$).

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Effect of medicinal plants and organic acid on growth performance of Ross broilers

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Introduction The prophylactic use of antibiotic (as growth promoters) in animal feeds has made intensive farming possible and improved feed conversion. In the presence of low levels of an antibiotic, resistant cells survive and grow which produces an antibiotic-resistant population. Consequently, the use of antibiotic for broilers has been limited. Therefore, a number of studies on alternative products that can aid promotion of growth, improved feed utilization, and maintenance of gut health have taken place (Hernandez *et al.*, 2004). Herbs and organic acid have received an increased attention as an alternative to antibiotics (Craig, 1999; Ricke, 2003). The main aim of the present research was to evaluate the efficiency of alternative antibiotic growth promoters on Ross broiler performance.

Materials and methods A total of 240 Ross male broiler chicks were fed in a completely randomized design with 4 replicate pens (15 birds 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+ medicinal plants (commercial mixture of medicinal plants, Digestrom) 450g/ton diet T₄= Control diet+ organic acid (commercial mixture of propionate, Formycine) 450g/ton diet. The 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 weight gain, average daily feed intake, feed to gain ration (or feed conversion ratio). The traits were measured for on individual chicks in the experiments. The data were analysed by a general linear model (GLM) by SAS programme. Multiple comparisons among different treatments were undertaken by Duncan multiple range test.

Results The results of analysis of variance for growth performance traits are shown in Table 1.

Table 1 Pair-wise comparisons* of different treatments for growth performance traits

Treatment	T ₁	T ₂	T ₃	T ₄	± SEM
Body weight, (g)					
21 d	593.99 ^b	697.27 ^a	615.51 ^b	609.89 ^b	10.21
42 d	1824.62 ^c	2045.03 ^a	1994.74 ^{ab}	1959.50 ^b	20.74
Average daily gain, (g)					
1 to 21 d	26.84 ^b	31.15 ^a	27.31 ^b	26.94 ^b	0.46
21 to 42 d	57.83 ^b	64.72 ^a	65.66 ^a	63.78 ^a	0.77
1 to 42 d	42.08 ^c	47.93 ^a	46.48 ^b	45.36 ^b	0.47
Average daily feed intake, (g)					
1 to 21 d	43.76 ^a	43.86 ^a	42.56 ^c	42.95 ^b	0.08
21 to 42 d	120.64	120.36	120.60	120.66	0.19
1 to 42 d	82.20 ^a	82.11 ^a	81.85 ^b	81.80 ^b	0.11
Feed: gain ratio					
1 to 21 d	1.70 ^a	1.42 ^c	1.59 ^b	1.59 ^b	0.02
21 to 42 d	2.10 ^a	1.88 ^b	1.85 ^c	1.91 ^b	0.02
1 to 42 d	1.90 ^a	1.65 ^c	1.75 ^b	1.72 ^b	0.01

*Treatments with similar letters were not statistically different from each other

Conclusions Supplementation with virginiamycin, medicinal plants and organic acid significantly ($p < 0.05$) improved body weight, average daily gain and feed:gain ratio broilers during the first 42 days when compared with treatment 1. The results obtained in the present research also revealed that for the traits of body weight as well as average daily gain and feed intake for the treatments 3 and 4 had lower values than those of the treatment 2. It can be, therefore, suggested that a higher level of medicinal plants and organic acid to be used to detect an accurate influence of the antibiotic alternatives on growth performance of broilers.

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Effects of vitamin E and C supplementation on performance and immune response of broiler chicks

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Introduction Vitamin E (VE) is known for its role as an antioxidant, protecting unsaturated bonds of cellular membrane phospholipids against free radical attack. VE also has been shown to be a requirement for normal development and function of the immune system. Although poultry are renal synthesizers of ascorbic acid, synthesis is inadequate under stressful conditions such as high environmental temperature, high humidity, high egg and meat production rate and parasite infestation. The main objective of this study was to assess the effects of various levels of dietary VE supplementation and Vitamin C (VC) in drinking water on performance, humoral and cell mediated immune response.

Materials and methods Commercial broiler males (Ross 208) were obtained from a local hatchery. One-day-old chicks were weighed and randomly allotted to dietary and water treatments. The basal corn-soybean meal diet was prepared according to NRC recommendations (1994), except that VE was omitted from the vitamin premix. Thirty-six pens of chicks (12 chicks per pen) were assigned to each of nine dietary treatments consisting of standard commercial starter and grower diets supplemented. The experimental design was a 3*3 factorial arrangement of three levels of dietary VE (0, 50, 75, mg dl-a-tocopherol acetate/kg of feed) and three levels of dietary VC (0, 500 and 1000ppm L-Ascorbic Acid in drinking water). At 26 days of age, three birds per pens were randomly assigned for lymphoblastogenesis assay in vitro. Blood samples (3ml/chick) were withdrawn with a EDTA syringe and diluted with equal volume of PBS. Blood from all birds in each pens was pooled and The peripheral blood lymphocytes were separated from the whole blood. Proliferative responses of lymphocytes were expressed as absorbance as described by Maslak and Reynolds (1995).

Results The results of the present study identified no significant differences in body weight gain in chicks fed supplemental VE to 42d. Proliferation of purified lymphocytes in mitogen-free cell culture medium (control) was affected by the level of dietary VE fed to chicks (data not shown). Chicks fed additional VC (1000ppm) exhibited a significant beneficial effect for body weight gain (42d), daily weight gain and feed consumption (21-42d). Mitogenic responses to PHA and Con A were significantly altered by VE. Proliferation of whole blood lymphocytes in the absence of mitogens was not affected with 50 and 75 IU/kg of added VE compared to the control. Chicks drank 500 and 1000 ppm of VC had significantly greater ($P < 0.05$) in vitro lymphocyte proliferative responses to ConA and PHA than those of chicks drank normal water. proliferation with mitogen and control (without mitogen) of whole blood lymphocytes in the mitogens PHA and Con A was not affected with VC. Lymphocyte proliferation in response to mitogens is correlated with the ability of the host to mount a cellular immune response. It has been suggested that differential reactivity to mitogens reflects either maturational or functional differences in the responsive lymphocytes. It was shown that many factors, including cell preparation, cell culture medium, and duration of the assay, might influence the effects of dietary factors on in vitro responses.

Table 1 Effect of vitamin E and C supplementation on the proliferation lymphocyte in purified lymphocyte and whole blood (OD in 540 nm)

Treatment	Purified lymphocyte			Whole blood		
	Without Mitogen (Control)	Mitogen		Without Mitogen (Control)	Mitogen	
		PHA	Con.A		PHA	Con.A
Vitamin E						
0	0.33 ^b	0.48 ^b	0.44 ^b	0.24	0.42 ^c	0.37 ^b
50	0.46 ^a	0.67 ^a	0.58 ^a	0.24	0.46 ^b	0.4 ^b
75	0.23 ^c	0.36 ^c	0.32 ^b	0.25	0.5 ^a	0.5 ^a
Vitamin C						
0	0.33 ^b	0.47 ^c	0.42 ^c	0.25	0.47	0.41
500	0.38 ^a	0.56 ^a	0.48 ^a	0.24	0.47	0.44
1000	0.32 ^b	0.49 ^b	0.45 ^b	0.25	0.45	0.43
SEM	0.007	0.008	0.006	0.012	0.008	0.008

Conclusions This study demonstrated that dietary VE improve the proliferative response of lymphocytes to polyclonal mitogens. VC supplementation (1000 ppm) improve body weight gain and cell mediated immune response, respectively. Moderate additions of combination VE (50 IU/kg) and VC affected the indices of immune response more than higher levels of supplementation. We hypothesize that moderate and high dietary levels of VE and VC may have different effects on the cellular free radical antioxidant balance, which results in different signal transduction events and activation states of the immune cells.

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Application of a duplex PCR approach for the specific and simultaneous detection of *Bifidobacterium* spp. and *Lactobacillus* spp. in duodenum, jejunum, ileum and cecum of broilers

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Introduction *Bifidobacterium* spp. and *Lactobacillus* spp genera are useful and important microbiota which affect poultry performance. These bacteria are natural inhabitants of the intestine of broilers. These bacteria have a unique hexose metabolism that occurs via the phosphoketolase pathway. *Bifidobacterium* spp. produces the vitamins B1, B2, B6, B12, nicotinic acid and folic acid. Enzymatic hydrolysis by *Bifidobacterium* spp. increases bioaccessibility of lipids and proteins. *Lactobacillus* spp. exhibit properties which include the ability to adhere to cells, persist and multiply and produce acids, hydrogen peroxide and bacteriocidal and bacteriostatic agents. The ability of *Lactobacillus* to inhibit bacterial pathogens including *E. coli* and *Salmonella* species and to influence parasitic infection including *Cryptosporidium parvum*, *Eimeria acervulina*, and *Giardia intestinalis* (Seidavi *et al.* 2008). The aim of this study was to develop a method for the direct and simultaneous detection of *Bifidobacterium* spp. and *Lactobacillus* spp. in the intestine by multiplex PCR.

Materials and methods Bacterial genomic DNA was extracted from the contents of gastrointestinal segments with the Wizard[®] Genomic DNA Purification Kit (Promega Corporation, Madison, WI, USA) with minor modifications. All oligonucleotide primers used in this study were synthesized by Metabion International AG. Sequences of the two PCR primer pairs for duplex PCR and size of expected amplification products are presented in Table 1. The GenBank program BLAST (<http://www.ncbi.nlm.nih.gov/BLAST>) was used to ensure that the applied primers were complementary with the target species but not with other species. Primers were compared with sequences in the GenBank, and none were found to have the exact same sequence as the non-targeted sequence. Different combinations of primer ratios were subsequently tested and the primer combination found to be optimal for duplex PCR was 0.28 μ M of each primer (Bif164-f, Bif662-r, LAA-f and LAA-r). The optimum Taq polymerase amount was determined by performing PCR for 0.5, 1, 1.5 and 2 U of Taq polymerase and 1 U of Taq polymerase produced high amplicon yields. The optimum Mg²⁺ concentration for duplex PCR was obtained by adding 1.2 mM MgCl₂. Finally, the optimum extension time was determined by performing PCR extension reactions for 45, 60, 90 and 120 s and an extension time of 60 s produced high amplicon yields. The PCR amplification mixture (25 μ l) consisted of 1 μ l of 25 ng DNA sample, 0.08 mM of each dNTP, 1.2 mM MgCl₂, 1 $\times$ PCR buffer, 0.28 μ M of each primer (Bif164-f, Bif662-r, LAA-f and LAA-r), 1 U of Taq DNA polymerase and 18.6 μ l ddH₂O. Amplification was performed on a thermocycler which initial denaturation was at 95°C for 3 min, followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 55.5°C for 45 s and extension at 72°C for 60 s, with a final extension at 72°C for 3 min.

Table 1 Primer sequences and size of PCR-amplified gene targets of two types of studied bacteria

Bacteria	Primer	Primer Sequence (5'→3')	Expected product size(bp)
<i>Bifidobacterium</i> spp.	Bif164-f	GGGTGGTAATGCCGGATG	510
	Bif662-r	CCACCGTTACACCGGGAA	
<i>Lactobacillus</i> spp.	LAA-f	CATCCAGTGCAAACCTAAGAG	286
	LAA-r	GATCCGCTTGCCTTCGCA	

Results All gastrointestinal samples tested including duodenum, jejunum, ileum and cecum showed positive results with the PCR assay for both *Bifidobacterium* spp. and *Lactobacillus* spp. There was no cross reactivity among these two closely related microorganisms. All primer pairs showed specificities only for their corresponding target microorganisms. In this investigation, two pairs of oligonucleotide primers were applied to simultaneously detect two different genera of useful bacteria by duplex PCR in a single tube. They are targeted at a species-specific region of the 16S rDNA. The specificity of the primers Bif164-f and Bif662-r for specific detection of *Bifidobacterium* spp. and of LAA-f and LAA-r for specific detection of *Lactobacillus* spp. has been reported. The primers can be used in gut samples with successful results. The genus-specific duplex PCR amplified 16S rDNA of the correct predicted size from the appropriate *Bifidobacterium* spp. and *Lactobacillus* spp. tested, but not from the other bacterial strains. When the two genus specific primer pairs were used individually, the same results were obtained as when the two reactions were run together as a duplex PCR. This technique complemented by microbiological technique to prove the validity of the PCR primers. Culturing of samples proved bacterial samples used in this study. Thus, the specificity of the PCR primers verified, and prevent to the detection of false positive.

Conclusion *Bifidobacterium* spp. and *Lactobacillus* spp. genera represent the probiotic cultures generally used in the food and poultry industry. The high throughput and cost-effective duplex PCR system developed in this study could provide a powerful supplement to conventional methods for more accurate risk assessment and monitoring of these useful bacteria in the poultry gut. Also in future, it must be developed approaches like real-time PCR for quantification of these bacteria.

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Investigation on relative population of *Salmonella* spp. bacteria in duodenum, jejunum, ileum and cecum of poultry using densitometry technique

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Introduction Salmonellosis is an infectious disease caused by *Salmonella* spp. that continues to plague human populations in both developed and third-world countries (Bohórquez, 2007). Conventional culture-based methods of detection that rely on enrichment of the sample and plating onto selective agar media may not be as sensitive as immunologic- or genetic-based rapid methods (Feng, 2001). PCR-based methods offer the advantages of high specificity and sensitivity. Some efforts have been made to reduce the time required and to increase the sensitivity and the accuracy of the methods to quantification of *Salmonella* in poultry samples (Mandrell and Wachtel 1999). This study was conducted in order to determine the relative population of *Salmonella* spp. in the duodenum, jejunum, ileum and caecum of chickens at different ages using densitometry.

Materials and methods Twenty four broilers were raised under conditions identical to those found in commercial broiler operations. DNA was extracted from duodenum, jejunum, ileum and cecum contents. Then PCR primers were optimized for detecting *Salmonella* spp. and all bacteria using specific and universal primers respectively. Amplified products were electrophoresed in 1.4% agarose gels containing ethidium bromide. A pUC Mix Marker 8 was used as molecular size marker. The relative population of *Salmonella* spp. to total gut bacteria was calculated by means of densitometry technique.

Results The population of *Salmonella* spp. relative to total bacteria in the different segments of the broiler gastrointestinal tract and different ages are shown in Figures 1 and 2. The relative populations of *Salmonella* spp. in the lower regions i.e. ileum and caecum were more than in the upper regions i.e. duodenum and jejunum. The highest and lowest relative population of *Salmonella* spp. were obtained at 4 and 30d of ages respectively. The results showed that the relative population of *Salmonella* spp. in duodenum, jejunum, ileum and cecum of broilers in 4d of ages were 0.28, 0.11, 0.16 and 0.91%. Also, relative population of *Salmonella* spp. in duodenum, jejunum, ileum and cecum of broilers in 14d of ages were 0.14, 0.18, 0.15 and 0.36%. Also, The highest and lowest relative population of *Salmonella* spp. in 30 day of ages obtained in ileum and duodenum of broilers (0.17 and 0.0%) respectively. Since these segments function differently and provide different environments, it is expected that different types of bacteria would colonize them. Also it is suggested bacteria population are transferred among various gut segments as animal aged.

Figure 1

Figure 1 *Salmonella* spp. population relative to total bacteria in different segments of broiler gut (%).

Figure 2

Figure 2 *Salmonella* spp. population relative to total bacteria (average counts for all segments) in different ages (%).

Conclusion Enzmann *et al.* (1999) reported densitometry technique have high efficiency and precise in mitochondrial DNA quantification relative to spectrophotometer approach. Zhang *et al.* (2007) calculated quantities of immunoglobulin proteins, bovine serum albumin, casein, and beta-lacto globulin in dairy products. They confirmed this approach is more precise and efficient than conventional methods. The densitometry based on PCR protocol used in this work quantified the population of *Salmonella* spp. relative to total bacteria in intestinal samples as efficiently. The results from the densitometry protocol were obtained a few hours after sample processing began. Furthermore this reduction in quantification time represents a true advantage compared to the traditional culture and other rapid methods in food and gut microbiology.

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Relative frequency alteration of *Escherichia coli* in broiler intestine

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Introduction *Escherichia coli* is a commensal bacterium of the gut microflora of the chicken. *Escherichia coli* infections are responsible for significant economic losses in the poultry industry world-wide. The pathogenesis and the role of virulence factors are not yet fully elucidated, although considerable progress has been made in recent years to establish the mechanisms of pathogenesis. *Escherichia coli* strains cause a number of diseases in domestic poultry, ultimately leading to disease and death, or to a decrease in egg and meat production or condemning of carcasses. On the other hand, our knowledge about the composition of the gut flora and microbial ecology of the gastrointestinal tract is still limited. Previous investigations have mainly used culture-dependent approaches. Studies on the composition of the intestinal flora of chickens date back to 1901 (Rahner, 1901) and were continued in the 1940s (Shapiro and Sarles, 1949), but comprehensive surveys that attempted to culture as many of the intestinal bacteria as possible were not carried out until the 1970s (Barnes *et al.* 1972; Salanitro *et al.* 1974). Such studies are technically difficult since strict anaerobic conditions have to be maintained during isolation and biochemical differentiation of the bacteria. It is well recognized that many bacteria have not been cultured yet in the laboratory because their growth requirements are still unknown. Recent molecular studies have yielded more detailed insight into the composition of the microbial community of this ecosystem (Zhu *et al.* 2002). The objective of this study was to develop a PCR based method for rapid quantification of *Escherichia coli* and investigation on its relative frequency in duodenum, jejunum, ileum and cecum of broilers.

Materials and methods DNA from gastrointestinal contents extracted as described by Seidavi *et al.* (2008). The specific detection of the *Escherichia coli* species was based on PCR amplification of the 16S rRNA gene using oligonucleotide primers ECO-f GACCTCGGTTTAGTTCACAGA and ECO-r CACACGCTGACGCTGACCA which PCR product size was 585 bp. Specific bands related to *Escherichia coli* obtained in duodenum, jejunum, ileum and cecum of broilers. Relative frequency alteration of *Escherichia coli* calculated by means of Gel-Proc Analyzer software for each age in different intestine segments and each intestine segment in different ages. Relative frequency is showed alteration proportion of this bacterium in various ages and segments. The linear regression with extrapolation method is selected for investigation on relative frequency alteration of *Escherichia coli*, since this method had the lowest variance. Obtained data from densitometry approach is entered and proceed using excel software.

Results In the rearing period, the highest relative frequency of *Escherichia coli* occurred in the cecum (76.47%) and the lowest relative frequency of *Escherichia coli* in the duodenum (0.0%) Relative frequency of *Escherichia coli* consists 62.72, 0.47 and 36.79% at 4, 14 and 30 day of ages. Other results are summarized in Tables 1 and 2. Today, accessing to a method not based on microbial culture is necessary for quantification of data related to bacteria populations, since PCR can detect target sequences and does not require cell growth. However microbial culture depended to target bacteria growth (Bjerrum *et al.* 2006).

Table 1 Relative frequency of *Escherichia coli* for each age in different intestine segments

Intestine Segment▶	Duodenum	Jejunum	Ileum	Cecum
Age (day)▶ 4	0.0	0.03	33.69	62.67
14	0.0	0.002	0.002	99.43
30	0.0	0.0	0.002	99.71

Table 2 Relative frequency of *Escherichia coli* for each intestine segment in different ages

Age (day)▶	4	14	30
Intestine Segment▶ Duodenum	0	0	100
Jejunum	99.94	0.05	0
Ileum	99.50	0.001	0
Cecum	51.40	0.61	47.97

Conclusion From obtained results is showed that relative frequency of *Escherichia coli* is different in duodenum, jejunum, ileum and cecum of broilers. Furthermore, this bacteria profile is altered at different rearing periods. Thus it is recommended that microbial flora studies perform PCR based methods for investigation on effects of factors such as diet ingredients, diet processing, enzyme, probiotics, environment etc on relative frequency of bacteria.

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Effects of different levels of canola meal on egg quality

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Introduction In recent years there has been increasing interest in cultivating canola in tropical areas. This product is used to supply edible oil for humans, and also as a high quality protein source for poultry (Baker and Chang 1992), soybean meal has been replaced by canola meal and there were some negative and positive effects on egg production, egg quality, egg shell quality (Roth-maier1999) In the present study, investigating the effect of canola meal on egg quality, different levels of soybean meal were replaced by canola meal in laying hen diets.

Materials and methods 96, 60 weeks old layer hens, Hy-line W-36 variety were used in a completely randomized design with 4 replicates in 3 periods (28 days per period) for investigating the soybean meal replacement by canola meal at 6 levels (0, 20, 40, 60, 80, 100%) based on crude protein, on egg quality. The data for Haugh unit, yolk colour, specific gravity, egg weight, egg production and egg taste were analysed by SAS(8.02)

Results The result of this experiment are summarised in table 1. The effects of different levels of canola meal on egg production, Haugh unit, yolk colour and egg taste were not significant ($P>0.05$). The specific gravity for 100% replacement treatment was significantly lower in comparison with other treatments ($P<0.05$). Egg weight has been effected by replacement of high levels of canola meal and it was significantly lower in comparison with control treatment ($P<0.05$).

Table 1 Ingredients of experimental diets

treatment	Cor n %	Soybean meal %	Canola meal %	DCP %	Oyster shell %	Soybean oil %	DLmet %	L- lys%	Salt %
0	67	18.12	0	1.16	10.87	1.93	0.09	0.02	0.31
20	65.5	14.64	4.55	1.14	10.82	2.38	0.08	0.08	0.31
40	64	11.5	9.11	1.12	10.77	2.82	0.08	0.14	0.32
60	62.5	7.67	13.66	1.1	10.72	3.27	0.07	0.2	0.32
80	61	4.19	18.22	1.09	10.68	3.72	0.07	0.26	0.32
100	59.2	0	23.69	1.06	10.62	4.26	0.06	0.33	0.32

Table 2 Effects of dietary treatments on egg weight, egg production, specific gravity, Haugh unit, Yolk colour and Egg taste.

treatment	Egg production	Egg weight	Yolk colour	Haugh unit	specific gravity	Egg taste
0	72.83	62.46 ^{ab}	7.94	82.7	1.08 ^a	5.86
20	73.83	62.7 ^a	8.1	83.43	1.08 ^a	6
40	74.92	61.44 ^{bc}	8.11	84.59	1.079 ^a	5.59
60	73.5	61.14 ^c	8.14	82.02	1.079 ^a	5.59
80	75.2	60.99 ^c	8.07	81.33	1.078 ^{ab}	6.14
100	73.58	61.01 ^c	7.97	84.9	1.075 ^b	5.63
SE	1.29	0.39	0.142	1.27	0.001	0.61

[†] Means by the same letter within columns are not significantly different at $P=0.05$ (Duncan's).

Conclusions It has been suggested before that high level of sulphur ion in canola meal has negative effects on calcium absorption, and also high levels of phytate in this product could decrease calcium and phosphorous absorption and deposition in egg shell and decrease egg specific gravity. High levels of phytate and crude fibre in this product decrease nutrient digestibility and result in decrease of egg weight (Summers *et al* 1988). This study showed that replacement of soy bean meal by canola meal could have economical saving in laying hen diets but because of mentioned reasons the suitable level for replacement could be debated and needs further investigation.”

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Modelling and optimising early performance for broiler chicks

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Introduction Several methods have been introduced to estimate the optimum level of dietary nutrients such as metabolisable energy (ME), crude protein (CP), and lysine (Lys) in broiler chicken production. Performance optimisation is usually measured as maximising body weight gain and minimising adjusted feed conversion ratio (Adj FCR). One useful method is to model a system that requires an explicit mathematical input-output relationship. Group method of data handling-type neural network (GMDH-type NN) and genetic algorithm (GA) is used to model and optimise an output in an imprecise environment (Yao, 1999). The purpose of this study was to apply the GMDH-type NN and GA methods to provide an optimised formula for broiler chicken performance based on the dietary level of ME, CP, and Lys.

Materials and methods Six hundred and forty d-old male broiler chicks (Ross 308) were assigned to eighty units of brooder batteries with 8 chicks per unit. The birds were fed a commercial starter diet (3,200 kcal of ME/kg, 23% CP, and 1.3% of Lys) till d 5. A factorial design experiment with four levels of ME (2900, 3000, 3100, and 3200 kcal/kg), four levels of CP (17, 20, 23, and 26 %), and five levels of Lys (1, 1.1, 1.2, 1.3, and 1.4 %) was used to provide eighty dietary treatments. The trial period started on d 5 and continued to d 20. The mean body weight gain (MBWG) and feed consumption of each unit were measured. Eighty data lines consisted of ME, CP, and Lys dietary levels as inputs, and the MBWG or Adj FCR as an output. The experimental data were imported to a GEvoM programme to generate polynomial equations (Ahmadi *et al.*, 2008; Nariman-Zadeh *et al.*, 2005). The GA was used to optimise polynomial equations and to find the optimum nutrient requirements for achieving maximum MBWG and minimum Adj FCR.

Results The corresponding polynomial equations produced to develop the MBWG model with goodness-of-fit criteria, R^2 and root mean square error (RMSE) were as follows:

$$y_1 = 171.272 + 5.732CP + 46.216Lys - 0.771CP^2 - 265.973Lys^2 + 30.207CP.Lys$$

$$y_2 = -0.000013 - 0.019839ME - 0.000002Lys + 0.000039ME^2 + 0.000014Lys^2 + 0.018739ME.Lys$$

$$MBWG = -0.000045 + 0.346757y_1 - 0.173136y_2 - 0.002605y_1^2 - 0.001864y_2^2 + 0.006698y_1.y_2$$

$$R^2 = 0.68 \quad RMSE = 25.5$$

The corresponding polynomial equations produced to develop the Adj FCR model with goodness-of-fit criteria, R^2 and RMSE were as follows:

$$y_1 = 2.319 - 0.060CP + 0.362Lys + 0.0022CP^2 + 0.321Lys^2 - 0.054CP.Lys$$

$$y_2 = 0.0000010 + 0.0014879ME + 0.0000006Lys^2 - 0.0000003ME^2 + 0.00000002Lys^2 - 0.0000056ME.Lys$$

$$Adj \ FCR = 45.087 + 2.640y_1 - 58.845y_2 + 0.692y_1^2 + 19.850y_2^2 - 2.424y_1.y_2$$

$$R^2 = 0.58 \quad RMSE = 0.08$$

The results of optimization, ME, CP, and Lys requirements for maximization of MBWG or minimization of Adj FCR are shown in Table 1.

Table 1 Optimum dietary nutrient requirements for broiler chicken performance during d 5 to 20

	ME (kcal/kg)	CP %	Lys %
MBWG	3158	25.87	1.37
Adj FCR	3036	24.97	1.38

Conclusion This method of data mining may be used to model and optimise broiler performance based on dietary nutrients. More data of this type is needed to more accurately model and optimise performance.

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Modifying egg fatty acid content by supplementation of laying hen diets with palm olein oil (POO)

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Introduction Palm oil is the most abundant of all oils produced globally. It is very high in saturated fatty acids specifically palmitic acid, but other fatty acids (monounsaturated (MUFA) and polyunsaturated) are presented at low concentrations. In the processing plant some high amount of oleic acid with some other unsaturated fatty acids are extracted and marketed as Palm olein oil, and used to reduce blood or egg cholesterol (Rievelles *et al.*, 1994). The objective of this study was to determine the optimum level of dietary palm olein oil required to enrich the mono-unsaturated fatty acid content of yolk, egg cholesterol and antibody titre.

Materials and methods Eighty four 26-wk-old white Hy-line W-36 laying hens were randomly assigned to 4 diets, with 3 replicates of 8 layers each. The experiment was conducted over a period of 12 wks. The mash diets were provided iso-caloric and iso-nitrogenous (ME= 2820kcal/kg, Cp= 16.2%, Lys=0.84% Na=0.18%, Ca=3.70% and AP=0.39%) and contained 0, 1.5, 3, and 4.5% of Palm olein oil (POO). At the end of experimental period, 2 hens per replicate were randomly selected, weighed, and blood was withdrawn from wing vein. Blood cholesterol and antibody titre against Newcastle disease (ND), Infectious bronchitis disease (IBD) were measured. One egg was randomly selected from each replicate hens, yolk was separated and stored in -20 C for later analyses. The yolk fatty acid contents were determined via the Folch method by GC apparatus. The data were analysed with SAS version 6.12.

Results The results of yolk and blood cholesterol and antibody titres, are presented in Table 1. The results of egg yolk fatty acid content and ratios of SFA/PUFA and omega-6/omega-3 are shown in Table 2.

Table 1 The effect of dietary palm olein oil on antibody titer, yolk and blood cholesterol of hens.

Performance	Dietary palm olein oil content (%)				SEM
	0.0	1.5	3.0	4.5	
Yolk cholesterol(mg/g)	12.95	13.20	13.55	13.75	0.65
Blood cholesterol(mg/dl)	143.00 ^b	157.67 ^{ab}	161.00 ^a	162.33 ^a	9.96
IBD titre	6812	6075	5590	5520	742.78
ND titre	8.67	8.33	8.33	8.67	0.67

^{a-b} Values within a row with no common superscripts differ significantly(P<0.05).

Table 2 The effect of dietary palm olein oil on yolk fatty acid contents and the ratio of SFA/PUFA and ω -6/ ω -3 fatty acids.

Fat content (%)	Dietary palm olein oil content (%)				SEM
	0.0	1.5	3.0	4.5	
Fatty acid					
Palmitic acid	34.92	34.13	32.78	29.99	1.65
Stearic acid	8.89	8.48	8.50	8.66	0.32
Oleic acid, n-9	39.96 ^c	40.06 ^c	43.13 ^b	46.00 ^a	0.707
Linoleic acid, n-6	10.24	10.49	10.51	10.39	0.42
Linolenic acid, n-3	0.50	0.56	0.47	0.46	0.154
Arachidonic acid, n-6	1.34	1.46	1.36	1.23	0.127
Eicosapentanoic acid, n-3	0.157	0.123	0.107	0.102	0.06
Docosahexaenoic acid, n-3	0.031	0.024	0.023	0.029	0.002
Σ n-3 fatty acids	0.69	0.62	0.60	0.58	0.005
Σ SFA ¹	43.24	41.35	41.14	38.95	2.28
SFA/PUFA ²	0.81	0.79	0.78	0.77	0.08
ω -6 / ω -3	18.01 ^b	19.43 ^a	19.63 ^a	19.75 ^a	0.28

a-c Values within a row with no common superscripts differ significantly(P<0.05).

1- SFA: saturated fatty acid, 2- PUFA: polyunsaturated fatty acid

Conclusion The different levels of dietary POO did not (P> 0.05) affect the saturated fatty acid (meristic, palmitic and stearic acid), ω -7 (palmitoleat), ω -6 (linoleate and arachidonate) and ω -3 (Linolenate, eicosapentenoate and Docosahexaenoate) content of egg yolk. The oleic acid (major fatty acid in the omega-9 family) was increased (P<0.05) as the level of POO increased in the diet. The SFA and SFA/PUFA ratio did not change with the level of dietary palm olein oil. The increase in dietary POO caused an increase in the ratio of ω -6/ ω -3 fatty acid and blood cholesterol (P<0.05). The yolk cholesterol, ND and IBD titre was not affected by dietary POO (P>0.05). It is concluded that the dietary POO level may affect the omega-9 fatty acid and the ratio of n-6/n-3 fatty acids of egg yolk.

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Analysis of inbreeding, effective population size and inbreeding depression in Iranian native fowls (Mazandaran Province)

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Introduction Mating of related individuals produces an inbred progeny, with an inbreeding coefficient that is one-half the additive relationship between its parents. One of the consequences of inbreeding is reduction of the mean phenotypic value or inbreeding depression, particularly for reproductive and fitness characters. Another consequence of inbreeding is reduction in additive genetic variance within lines or increasing homozygosity and higher risk for the incidence of lethal or deleterious recessive alleles. In recent years many studies show that inbreeding reduces reproduction and fitness capacity, for example Inbreeding depression in reproductive and productive traits has been reported by Flock *et al* (1993), Smith *et al* (1998), Klemetsdal (1998), Thompson *et al* (2000a,b) and Nwagu *et al* (2007).

The purpose of this study was to investigation recent trends in inbreeding in Iranian Native Fowls and relationship between increase in inbreeding and decreases in economic traits including body weight, age at first egg, egg production and egg weight.

Materials and methods A pedigree file of total 50,508 including 10458 cocks and 40050 hens (was born during 1988 to 2004) was investigated for the occurrence of inbreeding. Also body weight (BW), age at first egg (AFE), egg production (EP) and egg weight (EW) were analyzed for study the inbreeding effect on these traits. Inbreeding coefficients was included in the animal model as a linear covariate. The following animal models were employed:

for body weight: $y_{ijk} = \mu + s_i + g_j + h_k + bX_{ijk} + a_{ijk} + e_{ijk}$, for age at first egg: $y_{jk} = \mu + g_j + h_k + bX_{jk} + a_{jk} + e_{jk}$,

for egg weight: $y_{jk} = \mu + g_j + h_k + bX_{jk} + a_{jk} + e_{jk}$ and for egg production: $y_{jk} = \mu + g_j + h_k + bDIP_{jk} + bX_{jk} + a_{jk} + e_{jk}$, where y was observation of ijk individual, μ , s_i , g_j , h_k , DIP_{jk} , a_{ijk} , e_{ijk} were overall mean, sex, generation, hatch, days in production as covariate for egg number, random additive genetic effect and random error effect respectively, b and x_{ijk} were coefficient of partial linear regression and inbreeding coefficient deviation of ijk individual. Computations were performed using the DFREML package programmes of Meyer (2001). On the other hand the expected inbreeding according to the effective population size was predicted and compared with observed inbreeding during 14 generations. The first expected inbreeding was according to the number of parents (equation 1) and second one was according to the family size variance (equation 2):

$$\text{equation 1: } \frac{N_e}{N_m + N_f} \quad \text{equation 2: } \frac{N_e}{V_k + 2}$$

Results The overall mean inbreeding during 14 generation was 0.01246, so that a large proportion of population were inbred (26153 or 51.78%) with mean inbreeding of 0.02387. On the other hand the linear regression coefficients of BW, AFE, EN and EW on inbreeding was -0.457 g, 0.122 day, -0.218 number and -0.006 g per 1% increase of inbreeding respectively that its effect was significant only on egg number ($p < 0.001$).

Table and chart 1 Comparison of observed and expected inbreeding according to the effective population size: Obs: Rate of inbreeding according to the pedigree, Exp1: expected rate of inbreeding according to the number of parents and Exp2: rate of inbreeding according to the family size variance.

Gen	N _m	N _f	Ne1	Exp1	Ne2	Exp 2	Obs
0	94	574	323	0.000	344	0.000	0.000
1	89	617	311	0.002	284	0.001	0.000
2	87	659	307	0.003	445	0.003	0.000
3	99	654	344	0.005	456	0.004	0.000
4	111	717	384	0.006	250	0.005	0.000
5	95	683	334	0.008	269	0.007	0.000
6	102	705	356	0.009	290	0.009	0.001
7	87	588	303	0.010	124	0.011	0.004
8	85	656	301	0.012	178	0.015	0.003
9	84	609	295	0.014	123	0.018	0.004
10	81	637	287	0.015	149	0.022	0.008
11	79	555	277	0.017	77	0.025	0.004
12	94	645	328	0.019	160	0.031	0.025
13	86	634	303	0.020	63	0.034	0.027
14				0.022		0.042	0.048

Conclusions Inbreeding affected significantly only egg production with less heritability (0.16). It is clear that the expected inbreeding according to the number of parents (0.022) was less than observed (0.042). It means that this method can not predict inbreeding correctly because of non random mating. However, inbreeding according to family size variance was closer to observed inbreeding. This is because information from relatives in selection schemes is used (BLUP Animal Model) giving high co-selection probabilities, family size variance and increased rates of inbreeding during generations.

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White mulberry (*Morus alba*) fruit waste and its use in broiler nutrition

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Introduction The mulberry belongs to the genus *Morus* of the family Moraceae. There are 24 species of *Morus* and one subspecies, with at least 100 known varieties. One of the most important points about mulberry fruit is its sensitivity to environmental conditions during harvesting time and also the methods of harvesting. Because of these sensitivities, there is a lot of waste in the production of mulberry fruit. In the animal feed industry, agricultural by-products are used in animal and poultry nutrition. However, there is no information about the use of mulberry fruit waste in poultry nutrition; therefore this experiment was carried out to investigate the effect of mulberry fruit in broiler nutrition.

Materials and methods According to the result of previous experiment, the metabolizable energy of mulberry fruit waste is similar to corn grain (AMEn= 13.62MJ/Kd DM), but its crude protein content is half of corn grain (CP=4.4%). As the price of corn grain is 2.5-3 times higher than mulberry fruit waste, we can use this material as a source of energy for poultry. A total of 400 day-old chicks were randomly assigned to 16 litter floor pens (4 replicate per treatment). Experimental groups consisted of 0, 5, 10 and 15 % white mulberry that were inserted in broiler rations. The diets were formulated according to Cobb 500 nutritional recommendations for broiler chickens. Feed and water were provided for ad libitum consumption. In the experimental period (7-42 days) feed consumption (g/d), mortality (%), weight gain (g/d) and feed conversion were measured weekly. Finally at the age of slaughter (42d) 2 chicks were selected randomly from each replicate and carcass quality, carcass weight, breast, leg, abdominal fat, liver, kidney and heart weights were measured.

All data were subjected to one- way analysis of variance. Statistical significances among treatment means were determined by the method of new multiple range test of Duncan (1995) when the F value was deemed significant at 0.05.

Results Using different levels of white mulberry fruits waste had no significant effect on feed intake, but the amount of feed intake in all white mulberry fruits waste group was higher than the control. Weight gains in white mulberry fruit waste groups were lower ($P<0.05$) than controls. Though not measured, it seems that the diets which included mulberry waste had a higher bulk density, and this maybe one of the reason of decreasing weight gain without changing in feed intake. Carcass, leg, breast, heart and abdominal fat were not affected by the level of mulberry fruit waste. Kidney weight percentage was increased by increasing level of mulberry fruits waste supplementation. This increasing in kidney can be related to high content of K in mulberry fruits waste. Increasing concentration of white mulberry fruit waste was associated with an increase in liver weight (%) which may be related to increase of liver activity.

Conclusion In conclusion mulberry fruit waste can be used in poultry diets as an energy source (because of its low price in comparison with corn). In this experiment the use of mulberry had negative effect on performance so we recommended that further study evaluate the mechanism of this effect and determined the best level of supplementation and in poultry nutrition specially, layer hens.

Table 1 The effect of different levels of mulberry fruit waste on broiler performance

White mulberry fruits waste (%)	Feed Intake(g/d)	Weight gain(g/d)	Feed Conversion	Liveability (%)	BW42	Production Index
0	107.32	52.55a	2.04c	96	2139a	239.44 a
5	110.49	42.29b	2.24b	98	2008a	209.03 b
10	111.43	48.88b	2.28b	98	1995a	204.35b
15	110.95	43.40c	2.56a	96	1776b	158.81c
SEM	0.87	0.95	0.05	0.68	39.42	8

Table 2 The effect of different levels of white mulberry fruit waste on broiler carcass characteristics

White mulberry fruits waste (%)	Carcass (%)	Leg (%)	Breast (%)	Kidney (%)	Liver (%)	Heart (%)	Abdominal fat (%)
0	64.33	20.85	21.59	0.557b	2.47c	0.48	1.08
5	64.36	19.80	21.57	0.625ab	2.65bc	0.49	1.04
10	63.52	20.58	21.15	0.711a	2.99ab	0.54	0.913
15	66.67	19.81	20.99	0.969a	3.25a	0.55	1.02
SEM	0.97	0.24	0.23	0.02	0.09	0.02	0.11

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Effect of various levels of full-fat sunflower seed on performance of broiler chickens

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Introduction Sunflower (*Helianthus annuus*L.) is one of the most widely cultivated oilseeds in the world and ranks third in importance as a source of vegetable oil. As an alternative to fats and oils, full-fat oilseeds such as soybean seed are used to replace the supplemented fats and oils in broiler diets. However, soybean seed has anti-nutritional factors such as trypsin inhibitors, which need further processing, thus increasing the cost of soybean seed. Among the various oilseeds available on the market, full-fat sunflower seed (FFSS) contains more ether extract (EE) and is available at a relatively low price. This experiment was conducted to study the effect of FFSS (that FFSS had 18% CP, 38% EE, 14.3% CF, and 3868 kcal/kg ME) on performance of broiler chickens.

Materials and methods In this study, 176 day-old male broiler chickens (Ross strain) were allocated to four treatments with four replicate (11 in each replicate) in a completely randomized design to evaluate the effect of FFSS on the performance of chickens for 7 weeks. Treatments were 0, 7, 14 and 21 percent of FFSS for starter (1-21 days) and grower phases (22-49 days). The diets of starter phase calculated to contain 20.86% CP and 3000 kcal of ME per kg of diet. They also contained 18.75% CP and 3000 kcal of ME per kg of diet for the grower phase. Body weight gain and feed consumption were recorded. Data for all parameters were subjected to an analysis of variance, using the general linear model procedure of SAS (SAS Institute, 2004).

Results Feed intake increased significantly ($p<0.05$) when increasing levels of FFSS was incorporated in the diet during the experiment except for 43-49 days of age (Table). Weight gain also increased significantly in the different stages of our experiment ($p<0.05$). Except for 1 to 21 and 1 to 49 days of age, feed conversion ratio (FCR) improved significantly ($p<0.05$).

Table 1 Effect of full-fat sunflower seed on performance parameters of broiler (1-49 days of age)

FFSS (g/kg)	Feed Intake (g/b)				Weight Gain (g/b)				Feed Conversion Ratio			
	1-21	22-42	43-49	1-49	1-21	22-42	43-49	1-49	1-21	22-42	43-49	1-49
0	823.6 ^b	2446 ^b	1019	4125.1 ^b	417 ^b	1117 ^b	350 ^b	1741 ^b	1.97	2.54 ^a	2.91 ^a	2.39
70	874.7 ^{ab}	2793 ^a	1089	4757.4 ^a	451 ^{ab}	1099 ^b	456 ^{ab}	1935 ^{ab}	1.94	2.48 ^a	2.38 ^b	2.45
140	903.6 ^{ab}	2950 ^a	1168	4985.6 ^a	503 ^a	1199 ^{ab}	450 ^{ab}	2096 ^a	1.79	2.19 ^b	2.59 ^{ab}	2.38
210	932.1 ^a	2809 ^a	1139	4762.9 ^a	497 ^a	1311 ^a	492 ^a	2155 ^a	1.90	2.14 ^b	2.31 ^b	2.22
SE	29.47	24.40	85.35	5.93	10.01	20.97	24.83	0.069	0.121	0.281	0.10	

^{a,b}Within the same column, means with different letters are significantly different ($p<0.05$).

Conclusions FFSS was proven as a good source of CP and ME in broiler diets. The results from the current experiment indicated that substitution of FFSS for corn, soybean meal up to 210 g/kg of diet had positive effect on performance parameters. These results are in accordance with those obtained by Elzubeir and Ibrahim (1991), who reported that unprocessed sunflower seed can be given to broilers at up to 225 g/kg of the diet with no adverse effects on performance. In contrast with these findings, Dagher *et al* (1980) observed that feeding 150 and 250 g/kg-1 FFSS to broilers depressed both body weight gain and feed intake. One possible explanation for this disagreement of results is that different sunflower varieties or cultivars varying in chemical composition were used in the experiments.

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Determination of apparent metabolizable energy of full-fat sunflower seed in broiler chickens

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Introduction Sunflower (*Helianthus annuus* L.) is one of the most widely cultivated oilseeds in the world and ranks third in importance as a source of vegetable oil. In our experiment, full-fat sunflower seed (FFSS) had 180 g/kg CP, 380 g/kg EE, and 143 g/kg CF. Among the various oilseeds available on the market, FFSS contains more ether extract (EE) and is available at a relatively low price. This high EE content contributes to a high ME per unit or high energy density of feed. The increased production and availability of hybrid FFSS coupled with its oil content make FFSS a potentially desirable ingredient in poultry feeds. In the last few years, unextracted whole seed has been used as a feed ingredient in poultry diets. This experiment was conducted to determine the apparent metabolizable energy (AME_n) of FFSS in broiler chickens.

Materials and methods One-day old male chicks of Ross strain were housed in floor pens, exposed to light for 24 h/d, and fed a standard broiler diet for 2 wk. Feed and water were provided *ad libitum*. FFSS was incorporated into the basal diets at 3 concentrations (70, 140, and 210 g/kg). The 4 experimental diets, which contained 3 g/kg chromium oxide as an indigestible marker, were evaluated in a balance trial to determine the ME content. On d 10, 80 birds were placed at random in 16 cages giving 4 replicates per dietary treatment. On d 15, the birds were starved for 4 hours and then received the experimental diets from 15 to 21 d of age. During the last 3 d, excreta samples from each cage were collected and stored at -20°C. After being thawed, excreta were homogenized, dried, and ground through a 1-mm screen. Diets and excreta were analysed for dry matter, CP, chromium oxide, and gross energy. Apparent metabolizable energy was calculated as follows:

ME (MJ/kg) = dietary gross energy × [1 - (diet Cr₂O₃ / excreta Cr₂O₃) × (excreta gross energy / diet gross energy)].

The correction of AME to zero nitrogen retention (AME_n) was based on a factor of 34.4 kJ/g of retained N (Hill and Anderson, 1958). The AME_n value of FFSS was calculated using the following equation: AME_n = (AME_n T - α × AME_n B) / b, where T is the test diet, α is the proportion of the basal diet in the test diets, B is the basal diet, and b is the proportion of FFSS in the test diets. Statistical analyses were performed by using the GLM procedures of SAS software (SAS Institute, 2004). Data were subjected to ANOVA to identify variation produced by inclusion level of FFSS; regression analysis was also used to establish dietary changes as a function of inclusion level of FFSS.

Results Table shows AME_n data (MJ/kg) for the experimental diets and for FFSS using the difference technique described above. Increasing inclusion rate of FFSS increased the AME_n of the diets numerically but this effect was not significant. The AME_n (MJ/kg) of FFSS, calculated by difference (Table) ranged from 13.7 to 14.3 MJ/kg. The AME_n values obtained for the diets were regressed against the level of FFSS in the basal diet to estimate the AME_n content in FFSS. The equation derived by fitting a linear model was the following: $y = 12.394 + 0.0018X$ ($R^2 = 0.80$). An estimate of the AME_n of FFSS, obtained by extrapolation of this equation gave a value of 14.22 MJ/kg.

Table 1 Apparent metabolizable energy (AME_n)¹ of diets with increasing levels of full-fat sunflower seed (FFSS), and of FFSS determined by difference and regression analysis

Level of FFSS (g/kg)	AME _n of diets (MJ/kg)	AME _n of FFSS MJ/kg
0	12.42	-
70	12.51	13.74
140	12.60	13.68
210	12.82	14.31
SEM	0.164	

¹AME_n determinations were made based on 16 cages of 1 bird each.

Linear regression equation: $y = 12.394 + 0.0018X$; $R^2 = 0.8$ where $y = \text{AME}_n$ (MJ/kg) and $x = \text{dietary inclusion level of FFSS (g/kg)}$

In this experiment, the energy value obtained for FFSS was lower than the 18.70 MJ/kg and 17.67 MJ/kg reported by Rodriguez *et al* (1998) and Rodriguez *et al* (2005), respectively. This difference may be related to crude fat content because the sunflower seed they used had a higher amount of crude fat (473 and 444 g/kg respectively).

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Growth performance of broilers consuming wheat- or barley-based diets with or without enzymes

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Introduction Cereal grains, such as wheat, have been regarded as one of the most affordable ingredients to supply dietary energy for fast growing broilers. However, broilers compete with humans for wheat to satisfy their nutritional needs and also they cannot effectively utilise wheat due to the limited ability of their gut enzymes to utilise dietary fibre. The efficiency of wheat utilisation can be increased by adding exogenous enzymes into cereal based diets for these birds (Annison and Choct, 1993). These enzymes can also reduce fermentation in the small intestine and so help maintain the gut health. Therefore, we compared the effect of adding a commercial fibrolytic enzyme to wheat and barley-based diets on the utilisation of either diet by broilers from 0 to 35 days of age.

Materials and methods Two sets of four iso-nitrogenous (213-221 g CP/kg) diets with 12.6-13.6 MJ ME /kg were prepared by mixing (per kg diet) up to 650g coarsely ground wheat (A) or barley (C) with other ingredients in the absence (A and B) or presence of an enzyme (E= β xylanase) (A+E= B and C+E=D). Other ingredients were soybean meal, sunflower oil, sodium chloride, calcium mono phosphate, calcium carbonate and vitamin mineral premix. For the B and D diets, the enzyme was thoroughly mixed first with a portion of the diet which was then remixed with the relevant diet. One hundred and fifty six day old female Ross 308 chicks were transported from a commercial hatchery to the University Farm. The chicks were identified by using leg rings, weighed and divided into 12 experimental units of 13 chicks each where each unit was balanced for initial chick weight. Each chick unit was randomly housed in a circular pen on concrete floors covered with wood shavings in a pre-heated room at 35°C. This room was set for recommended light durations and each pen was equipped with a brooder, feeder and water container. Each of the 4 diets was allocated to 3 pens of 13 chicks each according to a completely randomized design. The brooding temperature was decreased weekly by 3°C until it reached 22°C. These chicks were offered *ad libitum* water and relevant starter diets for 21 days and grower cum finisher diets for another 14 days to the age of 35 days. Weekly body weight per chick and feed consumption per pen was recorded and feed efficiency calculated. Chick health was monitored and feed samples collected for their chemical composition. For this paper, only broiler growth data involving both the starter and finisher diets were analysed using ANOVA on Minitab to compare the effect of these diets on the broiler body weight at each week. The means of these diets in each week were compared by using Tukey test at $P < 0.05$.

Results The mean body weights (BW) of broilers at various days of age are summarized in Table 1. The birds continued to increase their diet intake with the increase in their age and so maintained a healthy growth. The BW at day 0 did not differ ($P > 0.05$) between diets confirming that the initial bird distribution was uniform across the diets. While the diets caused significant differences in BW at 7, 14, 21 and 35 days of age ($P < 0.05$), they did not differ at 28 days of age ($P > 0.05$). The impact of wheat and barley based diets was variable depending upon the age of these birds. The mean BW weight of birds consuming wheat based diets was greater than those on barley based diets showing significance at days 7 and 14 ($P < 0.05$). While, the overall effect of enzymes on BW was non significant at all days of age, the broilers had greater BW for the presence than the absence of enzyme in barley-based diets at 21 to 35 days of age ($P < 0.05$ for diet C v D).

Table 1 Mean body weight (g) of broilers fed wheat or barley based diets with or without enzymes at different days

Diets	Days →	0	7	14	21	28	35
A (Wheat)		35.7	164 ^{ab}	413 ^{ab}	808 ^a	1309 ^a	1829 ^{ab}
B (A+E)		36.0	175 ^b	422 ^b	790 ^a	1290 ^a	1819 ^a
C (Barley)		36.5	158 ^a	387 ^{ac}	746 ^b	1231 ^b	1680 ^c
D (C +E)		36.2	154 ^a	402 ^a	805 ^a	1300 ^c	1849 ^b

E= Enzyme; The figures with different superscripts in each column were significantly different at $P < 0.05$

Conclusions The birds had greater body weights for wheat than barley based diets at most days of age. However, enzyme addition appeared to have greater effect on body weights when barley plus enzyme was compared with barley without enzyme at 21, 28 and 35 days of age. The study shows a potential in enzymes to improve the utilisation of barley based diets in order to improve bird performance and so reduce the cost of broiler production.

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Effects of antibiotic and probiotic supplementation to diets containing fat on broiler performance

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Introduction The use of supplementary fat in commercial poultry diets has been wide-spread since the 1960s. In addition to their value as a dense source of energy, supplemental fats are an excellent source of essential fatty acids and enhance the absorption of fat soluble vitamins. The efficiency of nutrient digestion in poultry largely depends on the microorganisms which live naturally in its digestive tract (Apajalahti *et al.* 2003). It has been reported that intestinal microflora influences the absorption of fats (Pesti, 2002). Today, antibiotics and probiotics are used for manipulating the gut microflora in poultry production and act as growth-promoting agents. Thus, using these growth promoters (GP) will probably affect nutrients absorption, especially of dietary fats. The aim of this study was to evaluate the effects of supplementary antibiotics and probiotics in diets containing different levels of fat on broiler performance

Materials and methods Three hundred and sixty one-day old Ross broiler chicks were used in a 2×3 factorial arrangements with 2 levels of soy oil (3 and 6% of diet) and 3 levels of growth promoter (No-GP, antibiotic and probiotic), with 4 replicates and 15 birds per replicate in a randomized complete block design. Flavomycin (400g/t) and protexin (500g/t) were added to diet as growth promoter. Diets were iso-energetic and iso-nitrogenous and sand were used as inert to diets containing high level of oil to adjust their metabolisable energy. Feed and water were provided ad-libitum and a 24h lighting program was followed throughout the 42 days experiment. Feed intake and Body weight gain were recorded weekly and feed consumption ratio was calculated. Blood samples were collected randomly from two birds per each replicate at 35days of age. The blood samples were assayed for cholesterol, HDL, LDL, VLDL and triglycerides. All collected data were analysed using General Linear Models procedure of SAS (SAS, 2004), and means were compared by Duncan multiple range test.

Results The effects of fat levels, growth promoters on broiler performance and blood biochemical properties are presented in Table 1. Addition of probiotics to diets decreased feed intake and weight gain ($P<0.05$). Birds fed diets supplemented with probiotics had the lowest blood cholesterol and LDL. The probiotic effectiveness on broiler performance was related to levels of fat in diets and diets containing probiotic + 6% fat resulted the lowest weight gain ($P<0.05$).

Table 1 The effects of treatments on performance and blood biochemical properties of broiler chicks

Variables	Performance			Blood biochemical properties(mg/dl)				
	FI(g)	WG(g)	FCR	TG	Cholesterol	HDL	LDL	VLDL
Fat (%)								
3	3815.3 ^a	2203.1 ^a	1.75 ^b	71.9	162.1 ^b	85.2 ^b	66.8 ^b	14.2
6	3674.6 ^b	2022.6 ^b	1.82 ^a	74.1	197.7 ^a	94.4 ^a	86.7 ^a	14.8
SE	24.7	43.6	0.02	4.4	2.2	1.6	1.4	0.9
GP								
No-GP	3840.6 ^a	2110.0 ^b	1.76	71.6	179.8 ^b	60.3 ^{ab}	72.8 ^b	14.2
Antibiotic	3893.4 ^a	2221.5 ^a	1.81	69.0	195.3 ^a	96.0 ^a	91.4 ^a	13.7
Probiotic	3500.9 ^b	2007.0 ^c	1.79	78.4	164.6 ^c	82.9 ^b	66.1 ^c	15.6
SE	53.5	30.3	0.02	5.3	2.8	1.9	1.7	1.1
Fat(%) × GP								
3 × No-GP	4028.6 ^a	2233.9 ^a	1.77 ^b	74.2 ^{ab}	164.6 ^d	87.1 ^b	60.4 ^d	14.7 ^{ab}
3 × Antibiotic	3883.9 ^a	2216.4 ^a	1.75 ^b	77.2 ^{ab}	174.8 ^{cd}	93.3 ^{ab}	83.0 ^b	15.3 ^{ab}
3 × probiotic	3533.5 ^b	2159.1 ^a	1.73 ^b	64.3 ^b	146.9 ^e	75.2 ^c	57.0 ^d	12.8 ^b
6 × No-GP	3652.6 ^b	1986.1 ^b	1.8 ^b	68.9 ^b	195.1 ^b	93.6 ^{ab}	85.2 ^b	13.8 ^{ab}
6 × Antibiotic	3902.7 ^a	2226.7 ^a	1.77 ^b	60.8 ^b	215.9 ^a	98.8 ^a	99.8 ^a	12.1 ^b
6 × probiotic	3468.4 ^b	1854.9 ^c	1.89 ^a	92.4 ^a	182.2 ^c	90.7 ^{ab}	75.2 ^c	18.5 ^a
SE	75.7	42.9	0.03	7.5	3.9	2.7	2.4	1.6

^{a-d} Mean values with different superscripts on same column are significantly different ($P<0.05$).

Conclusions It was concluded that the supplementing diets containing fat with probiotics may have negative effects on broiler performance.

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Can medicinal plants with antimicrobial properties be replacement for antibiotics in broiler production?

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Introduction Antibiotics have been used for 50 years to enhance growth performance and to prevent disease in poultry production. Recently, most of the antibacterial growth promoters have been banned because the feeding of antibiotics is risky due not only cross-resistance but also to multiple resistances. Plants (especially herbs) have been used as food and medicinal purposes and some of them have played a significant role in maintaining human health and improving the quality of human life for thousands of years. Herbs or products containing plant extracts, essential oils or main components of the essential oil are among the alternative growth promoters that are already being used in practice. (Acamovic and Brooker, 2005; Ocak *et al.*, 2008). There is evidence suggesting that herbs, spices, and various plant extracts have appetizing, digestion-stimulating and antimicrobial properties. But, there is only limited evidence about whether the inclusion as a solid herb material would have the growth promoting effects in live birds. The aim of this study was to evaluate the antibacterial effects of dietary dry peppermint (*Mentha piperita* L.), Cumin (*Cuminum* L.), Milfoil (*Achellia millefolium* L.) and poley (*Teucrium Polium* L.) on the performance of broiler.

Materials and methods Three hundred and sixty one-old broilers (Arbor Acres) were randomly assigned to 6 treatment diets, with 4 replicates and 13 birds per replicate in a completely randomized design. The diets were isocaloric and iso-nitrogenous and contained 15, 3, 2 and 2 g/kg of dried herb of cumin, mint, Milfoil and poley, respectively. Two treatments with (flavomycin, 400g/t) and without antibiotic were considered as control groups. The diets were fed as mash and chicks were permitted free access to feed and water during the 42 days of experimental period. Continuous lighting was provided during the experiment. Body weight gain and feed intake were recorded weekly and feed conversion ratio was calculated on pen weight basis. All collected data were subjected to an analysis of variance, using the General Linear Model procedure of SAS (SAS Institute, 2004) and means were compared by Duncan's multiple range test.

Results The effects of treatments on broiler performance are presented in Table 1. Supplementation diets with antibiotic significantly ($p < 0.05$) improved body weight gain, and FCR in broilers during the 42 days rearing period when compared with other treatments.

Table 1 Effects of dietary inclusion medicinal plants and antibiotic on WG (g), FI (g) and FCR of broiler chicks.

Traits	Treatments						SEM
	Control	Antibiotic	Cumin	Mint	Milfoil	Poley	
Starter							
FI	397.6	404.6	396.1	399.6	387.7	385.5	5.75
WG	309.1 ^{ab}	319.1 ^a	308 ^{ab}	313.6 ^{ab}	297.2 ^{bc}	290 ^c	5.76
FCR	1.28	1.26	1.28	1.27	1.30	1.33	0.026
Grower							
FI	1196 ^c	1242.3 ^a	1200.7 ^{bc}	1222 ^{ab}	1209.3 ^{bc}	1195.9 ^c	7.53
WG	760.1 ^{bc}	776.4 ^a	753.5 ^c	767.4 ^{ab}	712.4 ^d	712.9 ^d	4.41
FCR	1.57 ^b	1.60 ^b	1.59 ^b	1.59 ^b	1.69 ^a	1.67 ^a	0.015
Finisher							
FI	2033.1 ^d	2163.5 ^b	2156.9 ^b	2196.4 ^a	2093 ^c	2157.5 ^b	10.94
WG	980.6 ^c	1030.1 ^a	982.2 ^c	1005.3 ^b	899 ^d	869.2 ^e	6.41
FCR	2.07 ^d	2.10 ^d	2.19 ^c	2.18 ^c	2.35 ^b	2.48 ^a	0.013
Total							
FI	3532.5	3589.8	3570.1	3601.1	3548.2	3504.5	38.15
WG	1936.7 ^c	2043.6 ^a	1982 ^{bc}	2008.5 ^b	1857.5 ^d	1821.3 ^c	11.06
FCR	1.79 ^b	1.75 ^b	1.80 ^b	1.79 ^b	1.91 ^a	1.92 ^a	0.019

^{a-d} Means in each row with different superscripts are significantly different ($P < 0.05$).

Conclusions It is concluded that medicinal plants had significant effect on broiler performance, but they can not be replacement for antibiotics. Mint leaves had most positive effects on weigh gain and feed conversion ratio than other studied medicinal plants. Further studies are needed to investigate the effects of different levels of mint, cumin, Milfoil or Poley on colonization and proliferation of microorganisms in the broiler intestine, and its performance.

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Effects of enzyme and probiotic supplementation to diets containing wheat on broiler performance

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Introduction Enzymes and probiotics are often used in feeding of poultry in intensive rearing systems. Beneficial effects of probiotics were observed in toxin neutralization, prevention of development and multiplication of specific bacteria, change in microbial metabolism and immunity stimulation. Administration of useful microorganisms as probiotic into diet usually cause enhancement of organic acids production such as lactic acid that in turn can reduce gastrointestinal pH and subsequent prevention of pathogen microorganisms such as salmonella to colonize the alimentary tract (Fuller, 1992). Exogenous enzymes have been shown to alleviate the adverse effects of high viscosity of digesta in the small intestine and to improve digestion (Petersen *et al.* 1999). Pervious studies demonstrate that simultaneously using probiotics and enzymes in broiler diets, improve their growth performance (Midili, and Tuncer, 2001). The objective of the present study was to examine the influence of probiotic (protexin) and enzyme (Natozyme) supplementation on the performance of broilers fed diets containing wheat.

Materials and methods Seven hundred and twenty male broiler chicks (Ross 308) were used in a 2×2×3 factorial arrangements with 2 levels of wheat (0 and 30% of diet), 2 levels of probiotics (0 and 300 g/ton) and 3 levels of enzyme (0, 100 and 200 g/ton), with 4 replicates and 30 birds per each replicate in a completely randomized design. Feed and water were provided ad-libitum and a 23:1 light: dark program was followed throughout the 6-week experiment. Feed intake and body weight gain were recorded weekly and feed consumption to weight gain ratio (FCR) was calculated. At the end of the experiment (week 6), two birds from each replicate were randomly selected, slaughtered and the weights of liver, caeca and abdominal fat were measured. All data were analysed using General Linear Model's procedure in SAS (SAS Institute, 1994) and means were compared by Duncan's test at 5% of probability

Results The effect of wheat, protexin and enzyme on broiler performance are shown in Table 1. The results showed that 30% wheat in diets decreased feed intake and body weight gain, significantly ($P<0.05$). Supplementation diets with Protexin significantly ($p<0.05$) improved feed intake, body weight and FCR, but addition of enzyme to diets had not significant effects on broiler performance. Relative weight of the abdominal fat significantly decreased in bird fed diets containing protexin, but protexin increased caeca weight ($P<0.05$). The interaction effect between enzyme × probiotic × wheat on body weight gain and FCR was significant. Supplementation wheat-soy based diets with probiotic and enzyme improved body wheat gain and FCR, significantly ($P<0.05$).

Table 1 Effect of different levels of enzyme and probiotic and wheat on the performance of broiler chickens

Factor	FI(g)	BWG(g)	FCR	Abdominal fat%	Liver%	Caeca%
Wheat (%)						
0	4747 ^a	2224 ^a	2.12 ^a	1.05	1.29	0.49
30	4725 ^b	2114 ^b	2.34 ^b	1.10	1.34	0.46
Enzyme(g/ton)						
0	4702	2007	2.33	1.07	1.33	0.47
100	4760	2061	2.21	1.06	1.25	0.45
200	4728	2055	2.27	1.09	1.27	0.51
Probiotic(g/ton)						
0	4677 ^b	2007 ^b	2.36 ^b	1.16 ^a	1.31	0.44 ^b
300	4798 ^a	2131 ^a	2.21 ^a	0.99 ^b	1.32	0.51 ^a
SEM	6.01	8.71	4.97	14.47	12.87	19.55

^{a, b} Mean values with different superscripts on same column are significantly different ($P<0.05$).

Conclusion It is concluded that the simultaneously using of probiotics and enzyme in wheat – soy based diets is a good way to obtain more economical benefits in broiler production.

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Effect of different levels of digestible lysine on performance, and blood parameters of male and female broilers in the starter period

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Introduction Lysine is the reference amino acid (AA) in the ideal AA ratios for chickens. Feed formulation based on digestible AA has been shown to increase weight gain and feed intake and improve body composition in broilers. Amino acid (AA) in most feed ingredients will not be totally digested, and knowledge of such efficiency is important in formulating diets and will be used to eliminate differences in absorption efficiencies due to feedstuff sources. This study was conducted to evaluate the growth performance and blood parameters of broilers fed various levels of DL (Digestible Lysine) supplemented in diets from day 1 to day 18.

Materials and methods Arian male and female broilers (1-18 days) by feeding practical diets formulating on digestible amino acids basis. A total of 240 male and female chicks were used (initial weight of 44±1 g). The birds were distributed in a completely randomized design, using six treatments and four repetitions, with 5 chicks per repetitions. These experimental chickens were kept in thermostatically controlled batteries. The experimental treatments consisted of a lysine-deficient basal diet that was supplement with L-lysine-HCl in order to contain six digestible lysine levels (0.68, 0.8, 0.92, 1.04, 1.16 and 1.28%). These diets were isocaloric, isonitrogenous and equal in electrolyte balance. All diets met an the Illinois recommended ideal amino acid ratio for all other amino acids. Feed and water supplied for *ad libitum* consumption. During the experiment feed consumption (FC), weight gain (WG) and feed conversion ratio (FCR) were measured weekly. Also, blood samples were collected at the end of the starter period. Data were analysed with SAS software in proc ANOVA and Duncan's multiple range test were used to compare treatments means. The results were showed this form: Mean ± SD (Standard Deviation)

Table1 Effects of digestible lysine levels on blood parameters

	DL (%)	Lys (nmol/ml)	Albumin (g/dl)	Uric Acid (mg/dl)	BUN (mg/dl)	Creatinine (mg/dl)
Male	0.68	70±1.10 ^c	0.90±0.02 ^c	15.10±0.07 ^f	6.61±0.07 ^e	0.78±0.00 ^c
	0.80	90±6.35 ^d	1.38±0.35 ^d	15.58±0.08 ^e	6.44±0.08 ^e	0.75±0.02 ^b
	0.92	140±7.34 ^c	1.70±0.00 ^c	16.21±0.09 ^d	5.66±0.17 ^d	0.73±0.01 ^b
	1.04	140±3.77 ^c	1.93±0.01 ^b	16.50±0.18 ^c	5.22±0.15 ^c	0.70±0.01 ^a
	1.16	150±5.23 ^b	2.40±0.01 ^a	17.11±0.17 ^a	4.00±0.20 ^b	0.71±0.01 ^a
	1.28	160±2.82 ^a	2.38±0.01 ^a	16.83±0.07 ^b	3.29±0.06 ^a	0.80±0.01 ^c
	Mean	125.01	1.78	16.22	5.20	0.75
	0.68	50±2.00 ^f	0.70±0.01 ^f	12.37±0.10 ^a	5.49±0.10 ^f	0.45±0.01 ^d
Female	0.80	70±1.09 ^e	1.12±0.01 ^e	12.72±0.17 ^b	5.10±0.14 ^e	0.41±0.01 ^c
	0.92	90±5.21 ^d	1.50±0.01 ^d	13.20±0.10 ^c	4.82±0.05 ^d	0.40±0.01 ^{bc}
	1.04	110±5.53 ^c	1.96±0.01 ^a	13.62±0.20 ^d	4.47±0.10 ^c	0.36±0.10 ^a
	1.16	120±4.12 ^b	1.90±0.00 ^c	14.10±0.12 ^c	4.00±0.10 ^b	0.49±0.01 ^e
	1.28	140±3.99 ^a	1.92±0.01 ^b	13.90±0.04 ^c	3.53±0.09 ^a	0.39±0.00 ^b
	Mean	96.67	1.52	13.32	4.57	0.42

Results Increase of digestible lysine level to 1.28% due to: maximum body weight, plasma lysine level, minimum feed conversion. There was the best of plasma albumin level and carcass N deposition in 1.16% and 1.04% of digestible lysine diet level for male and female respectively in the starter phases.

Table2 Effects of DE levels on feed intake, BW, FCR and carcass nitrogen

	DL (%)	Body Weight(g)	FCR	Feed Intake(g)	N.Carcass (g bird-1 day-1)
Male	0.68	370.71±6.54 ^d	2.08±0.03 ^e	772.92±17.21 ^d	0.86±0.01 ^e
	0.80	380.23±7.62 ^d	2.06±0.17 ^e	782.39±20.10 ^d	1.16±0.01 ^d
	0.92	415.27±12.91 ^c	1.75±0.01 ^d	726.74±23.82 ^c	1.16±0.01 ^d
	1.04	463.10±8.20 ^b	1.51±0.03 ^c	700.30±8.79 ^c	1.19±0.01 ^c
	1.16	485.58±8.14 ^a	1.31±0.03 ^b	637.42±21.50 ^b	1.24±0.02 ^a
	1.28	490.60±9.63 ^a	1.20±0.02 ^a	588.70±12.80 ^a	1.21±0.02 ^b
	Mean	434.25	1.65	701.41	1.37
	0.68	342.53±2.87 ^f	2.12±0.01 ^f	724.45±8.23 ^c	0.80±0.01 ^f
Female	0.80	358.30±8.56 ^e	2.01±0.01 ^e	717.54±18.17 ^c	0.92±0.01 ^e
	0.92	392.66±2.59 ^d	1.86±0.12 ^d	730.35±7.27 ^c	0.98±0.01 ^d
	1.04	405.77±8.47 ^c	1.60±0.02 ^c	649.32±1.73 ^b	1.20±0.01 ^a
	1.16	415.19±3.86 ^b	1.47±0.02 ^b	609.25±4.14 ^a	1.14±0.01 ^c
	1.28	426.02±3.59 ^a	1.41±0.14 ^a	600.68±6.97 ^a	1.16±0.01 ^b
	Mean	390.08	1.743	671.93	1.03

Conclusion This experiment supports the hypothesis that the different digestible lysine levels enhance protein utilization in broilers. All parameters as well were also improved with increase of digestible lysine levels and this suggests an effect of the amino acids utilization. Several possible mechanisms may account for the enhanced growth of chickens in response to additional dietary lysine. These include increased availability of lysine for protein synthesis, stimulated secretion of hormones such as glucagon, insulin and growth hormone which may consequently increase protein synthesis and feed intake.

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Dietary digestible lysine immune responses and carcass nitrogen of broiler chickens in starter period

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Introduction lysine is an essential amino acid that promotes normal growth by helping to maintain the proper protein balance in the body. Most vegetable protein sources used in poultry diet formulations are moderate to low in lysine contents; hence supplementation with lysine is inevitable in growing broilers to ensure rapid growth and optimum efficiency of feed utilization. Adequate supply of nutrients during the starter improves gut development and could result in long term improvements in feed utilization. In order to evaluate the effect of different digestible lysine dietary levels on growth and immune response in starter, this experiment was done.

Materials and methods 240 Arbor Acres broiler chicks (1-18 days) were used in a completely randomized design experiment (6 dietary, 4 replicates and 5 birds per each replicate). The levels of digestible lysine dietary were 0.68, 0.8, 0.92, 1.04, 1.16 and 1.28% (feeding practical diets formulating on digestible amino acids basis). All diets were formulated to meet NRC (1994) requirements and were formulated on a digestible amino acid basis with maintained ratios of dietary essential amino acids to lysine content. These diets were isoenergetic, isonitrogenous and equal in electrolyte balance (Na + K – CL). All diets met an ideal amino acid ratio recommended Illinois for all other amino acids. Feed and water supplied for *ad libitum* consumption. During the experiment feed conversion ratio (FCR) were measured weekly. Also, blood samples were collected to determine antibody titer (Titr), white blood cells (WBC), Lymphocyte (L), Heterophyle (H), immune index (H/L) and the antibody response to a nonpathogenic antigen sheep red blood cell (SRBC) on day 18. A linear model and Duncan's mean test were used to analyze data by applying SAS software.

Results different digestible lysine levels had a significant effect on FCR and nitrogen deposition. Immune response as well was also improved with increase of digestible lysine levels and this suggests an effect of the on amino acids utilization.

Table 1 Effect of different digestible lysine levels on immune response, FCR and nitrogen deposition

	DL(%)	WBC($\times 10^3/\mu\text{l}$)	H(%)	H/L	Titr(log2)	SRBC(log2)	FCR	N Deposition
Male	0.68	14300 $\pm$ 173 ^f	15 $\pm$ 2.24 ^f	0.37 $\pm$ 0.04 ^f	1.50 $\pm$ 0.01 ^f	1.54 $\pm$ 0.06 ^d	2.08 $\pm$ 0.03 ^e	0.86 $\pm$ 0.01 ^e
	0.8	15200 $\pm$ 189 ^e	19 $\pm$ 1.41 ^e	0.42 $\pm$ 0.02 ^e	1.92 $\pm$ 0.03 ^e	1.70 $\pm$ 0.06 ^c	2.06 $\pm$ 0.17 ^e	1.16 $\pm$ 0.01 ^d
	0.92	19000 $\pm$ 124 ^d	22 $\pm$ 0.43 ^d	0.47 $\pm$ 0.01 ^d	2.52 $\pm$ 0.02 ^d	1.78 $\pm$ 0.05 ^c	1.75 $\pm$ 0.01 ^d	1.16 $\pm$ 0.01 ^d
	1.04	25900 $\pm$ 90 ^c	27 $\pm$ 0.36 ^c	0.58 $\pm$ 0.01 ^c	2.58 $\pm$ 0.02 ^c	2.10 $\pm$ 0.11 ^b	1.51 $\pm$ 0.03 ^c	1.19 $\pm$ 0.01 ^c
	1.16	28000 $\pm$ 184 ^b	33 $\pm$ 1.07 ^b	0.62 $\pm$ 0.02 ^b	2.74 $\pm$ 0.03 ^b	3.12 $\pm$ 0.11 ^a	1.31 $\pm$ 0.03 ^b	1.24 $\pm$ 0.02 ^a
	1.28	41300 $\pm$ 160 ^a	39 $\pm$ 1.27 ^a	0.69 $\pm$ 0.02 ^a	2.88 $\pm$ 0.01 ^a	3.22 $\pm$ 0.10 ^a	1.20 $\pm$ 0.02 ^a	1.21 $\pm$ 0.02 ^b
	Mean	23950	25.83	0.52	2.36	2.24	1.65	1.37
	0.68	16800 $\pm$ 195 ^f	17 $\pm$ 1.18 ^f	0.38 $\pm$ 0.02 ^f	1.45 $\pm$ 0.01 ^f	1.50 $\pm$ 0.06 ^e	2.12 $\pm$ 0.01 ^f	0.80 $\pm$ 0.01 ^f
Female	0.8	19600 $\pm$ 259 ^e	20 $\pm$ 1.18 ^e	0.41 $\pm$ 0.01 ^e	1.70 $\pm$ 0.01 ^e	1.66 $\pm$ 0.06 ^d	2.01 $\pm$ 0.01 ^e	0.92 $\pm$ 0.01 ^e
	0.92	23800 $\pm$ 139 ^d	26 $\pm$ 0.63 ^d	0.52 $\pm$ 0.01 ^d	2.08 $\pm$ 0.01 ^d	1.73 $\pm$ 0.05 ^d	1.86 $\pm$ 0.12 ^d	0.98 $\pm$ 0.01 ^d
	1.04	26600 $\pm$ 135 ^c	30 $\pm$ 0.67 ^c	0.59 $\pm$ 0.02 ^c	2.56 $\pm$ 0.03 ^c	1.94 $\pm$ 0.11 ^c	1.60 $\pm$ 0.02 ^c	1.20 $\pm$ 0.01 ^a
	1.16	32200 $\pm$ 43 ^b	35 $\pm$ 0.76 ^b	0.66 $\pm$ 0.01 ^b	2.74 $\pm$ 0.02 ^b	2.27 $\pm$ 0.11 ^b	1.47 $\pm$ 0.02 ^b	1.14 $\pm$ 0.01 ^c
	1.28	44100 $\pm$ 211 ^a	42 $\pm$ 1.52 ^a	0.73 $\pm$ 0.01 ^a	2.78 $\pm$ 0.03 ^a	2.40 $\pm$ 0.11 ^a	1.41 $\pm$ 0.14 ^a	1.16 $\pm$ 0.01 ^b
	Mean	27183.33	28.33	0.55	2.22	1.92	1.743	1.03

Conclusion The results of this experiment support the hypothesis that the different digestible lysine levels enhance protein utilization in broilers. The influence of Lys was probably mediated by IGF-I (Conconi, 2001). High immune response is possibly due to increased protein availability for liver protein synthesis associated with immune response or antibody production. In the present study, increasing the dietary digestible lysine levels from 0.68 to 1.28% of the NRC-recommended requirement significantly increased immune response and nitrogen retention. These results concur with those previously obtained from chickens (Bons *et al.*, 2002). Supplementation of L-lysine also increased their antibody responses, with increasing circulating lymphocytes, monocytes, neutrophils, and humoral response. Understanding the nuances of nutrition and immunity is important for optimizing bird health and productivity, and will be an important contributor towards fulfilling the consumer's conflicting demands for more natural production and better animal welfare. Immune function, especially of lymphocytes, should be monitored when conducting experiments to determine nutrient requirements. Least-cost diets are not usually optimal for immunity because they deliver too much energy and are marginal in some nutrients. Experiments to date indicate that concentrations of some nutrients that give optimal growth and efficiency of feed utilization are inadequate for immunity. Among amino acids, the dietary concentrations that support maximal growth performance appear to be adequate for immunity for lysine, arginine, isoleucine and valine; but sulphur amino acids may be an exception (Klasing, 2007).

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Effect of prebiotic on performance of broiler chicks in low protein diets

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Introduction In view of the severe restriction or total ban on the use of antibiotics as growth promoters and therapeutic agents in poultry industry, probiotics and prebiotics have been suggested as alternatives to antibiotics (piray *et al*, 2007). Prebiotic are known as "a nondigestible feed ingredient" that beneficially affects the host by selectively stimulating the growth or activity of a limited number of bacteria in the colon (Gibson and Roberfroid, 1995). Prebiotics has a significant effect on body weight gain and feed to gain ratio (piray *et al*, 2007). The objective of this research was to study the effectiveness of adding prebiotic on broiler growth performance in low protein diets.

Materials and methods Two hundred forty day of hatch mixed sex broiler chickens of the Ross strain-308 were housed on litter-floor and allocated randomly to each of 6 treatments with 4 replicates (pens) each in CRD design. The environmental condition was according to the Ross guide book. All groups received starter diet contains 23% crude protein from days 1-10. Three groups received an experimental diet formulated to meet Ross guide nutrient requirements for crude protein (21% in grower diet from days 11-28 and 19% in finisher diet from days 29-42) and other nutrients with three levels of (0.0, 1.5 and 3.0 g /kg into the basal diets) Aspergillus meal prebiotic (Fermacto). It is the feed additive, derived from Aspergillus mycelium. The other 3 groups received a diet deficient in crude protein (19% in grower diet from days 11-28 and 17% in finisher diet from days 29-42) with the same three level of prebiotic. Feed and water were provided ad-libitum. Feed intake and body weight gain of chickens were recorded weekly and feed to gain ratio calculated as the unit of ate feed per unit of body weight gain (g/g). GLM proc of SAS was used for statistical analysis and least square means.

Results In finisher period (29-42 days) supplementation of Fermacto increased feed intake only in diet containing standard protein with 3 g/Kg Fermacto. However, difference between diet containing standard protein with 3 g/Kg Fermacto and three low protein diets was significant ($p < 0.05$). Addition of Fermacto into low protein diets did not improved feed intake in comparison to control. Addition of Fermacto into both standard and low protein diets increased weight gain but differences were not significant. Among low protein diets, highest value of weight gain observed in diet with 3 g/Kg Fermacto. Chicks fed low protein diet with 3 g/kg Fermacto did not show significant difference in weight gain in comparison to standard protein diets. Chicks fed low protein diet with 3 g/kg Fermacto had comparable feed conversion ratio (FCR) value with standard protein diets. Using Fermacto in standard protein diet had not significant effect on FCR but addition of 3 g/Kg Fermacto into low protein diet improved FCR significantly ($p < 0.05$).

Table 1 Mean feed intake, weight gain and FCR in chickens fed two levels of protein with three levels of Fermacto in finisher period.

Diets	Feed intake, g	weight Gain, g	FCR	Body weight, g
SP ¹ + 0 g/Kg Fermacto(control)	2000 ^{ab3}	1126 ^{abc}	1.78 ^{bcd}	2711 ^{ab}
SP+1.5 g/Kg Fermacto	1958 ^{bc}	11277 ^{ab}	1.74 ^{cde}	2614 ^{bc}
SP+ 3 g/Kg Fermacto	2060 ^a	11583 ^a	1.78 ^{bc}	2782 ^a
LP ² + 0 g/Kg Fermacto	1943 ^{bcde}	1026 ^{de}	1.90 ^a	2533 ^c
LP+1.5 g/Kg Fermacto	1946 ^{bcde}	1066 ^{bcde}	1.83 ^{ab}	2589 ^{bc}
LP+ 3 g/Kg Fermacto	1956 ^{bcd}	1101 ^{abcd}	1.78 ^{bcde}	2604 ^{bc}
SEM	0.0295	0.0271	0.0264	54

¹ Standard protein ² Low protein

³Means in each column with different superscripts differ statistically ($p < 0.05$).

Conclusions Generally, using Fermacto at high level (3g/kg) in standard and low protein diets improved weight gain and FCR of chicks numerically and statistically respectively. FCR and weight gain values were more affected in low protein diets containing Fermacto. This findings show potential using of Fermacto in some countries which could not formulate diets at protein level recommended by Ross company.

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Effect of prebiotic on lipid profile of broiler chicks

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Introduction Research on poultry genetics, feeding and management for BWG and FCR resulted in fast growth but decreased the quality of poultry products as modern fast growing broilers have been found to contain higher amount of abdominal fat (Chambers *et al*, 1981). Most recently considerable attention has been paid to test the potency of growth promoters on altering lipid metabolism, because, World Health Organization suggest that excess fat deposition is undesirable in human body. Recent report suggested that feeding of chicory beta fructans; a prebiotic reduced the serum cholesterol and abdominal fat of broiler chicken. The present study was undertaken to study the effect of *Aspergillus* meal prebiotic (Fermacto) on abdominal fat, serum total cholesterol, HDL and triglyceride levels in low protein diets.

Materials and methods Two hundred forty day of hatch mixed sex broiler chickens of the Ross strain-308 were housed on litter-floor and allocated randomly to each of 6 treatments with 4 replicates (pens) each in CRD design. Three groups received an experimental diet formulated to meet Ross guide nutrient requirements for crude protein (21% in grower diet from days 11-28 and 19% in finisher diet from days 29-42) and other nutrients with three level of (0.0, 1.5 and 3.0 g /kg into the basal diets) Fermacto. Fermacto is the feed additive, derived from *Aspergillus* mycelium. The other 3 groups received a diet deficient in crude protein (19% in grower diet from days 11-28 and 17% in finisher diet from days 29-42) with the same three level of Fermacto. A day prior to slaughter, blood samples were randomly collected via wing vein from 5 males of each treatment at 48 days of age. After slaughter, abdominal fat were measured in the dressed carcasses. Serum samples were analyzed for total cholesterol, HDL cholesterol and triglycerides in Imam Reza hospital biochemical lab. GLM proc of SAS was used for statistical analysis and obtaining least square means.

Results In recent study, supplementation of Fermacto decreased total cholesterol in diet containing standard protein with 1.5 g/kg Fermacto significantly ($p<0.05$). With addition of 1.5g/kg Fermacto to standard protein diet, the cholesterol value decreased from 148 to 125.8 mg/dl. However, in the diets containing low protein, addition of Fermacto had no effect on cholesterol level in comparison to control diet. Triglycerides level did not affected by supplementation of Fermacto in diets containing low protein. However, supplementation 3 g/kg Fermacto in standard protein diet decreased triglycerides level in comparison to control diet ($p<0.05$). Decreasing of HDL observed only in diet containing standard protein with 1.5 g/kg Fermacto in comparison to control diet ($p<0.05$), but in other treats did not show significantly differences. These observation show that serum lipids affected by Fermacto in standard protein diets but not in low protein ones. There was decreasing trend in percentage of abdominal fat pad with addition of Fermacto to diets containing standard protein. However, the diets containing low protein, addition of Fermacto with 1.5 g/kg decreased significantly ($p<0.05$) abdominal fat pad in comparison to diet containing low protein without Fermacto.

Table 1 Serum lipid and abdominal fat(% of live weight) content of broiler fed different levels of protein and Fermacto

Diets	Total Cholesterol(mg/dl)	Triglycerides(mg/dl)	HDL(mg/dl)	Abdominal fat	Body weight, g
SP ¹ + 0 g/Kg Fermacto(control)	148 ^{a3}	147.2 ^a	118.8 ^a	2.58 ^{ab}	2711 ^{ab}
SP+1.5 g/Kg Fermacto	125.8 ^c	133 ^{abcde}	108 ^{cde}	2.25 ^{abcd}	2614 ^{bc}
SP+ 3 g/Kg Fermacto	135.4 ^{bcde}	126.2 ^{bcde}	117 ^{abc}	2.14 ^{bcde}	2782 ^a
LP ² + 0 g/Kg Fermacto	138.6 ^{abcd}	145.6 ^{ab}	113 ^{abcd}	2.80 ^a	2533 ^c
LP+1.5 g/Kg Fermacto	140 ^{abc}	142 ^{abcd}	112 ^{abcde}	2.12 ^{bdec}	2589 ^{bc}
LP+ 3 g/Kg Fermacto	140.2 ^{ab}	143.4 ^{abc}	118 ^{ab}	2.36 ^{abc}	2604 ^{bc}
SEM	3.9476	6.8946	3.2660	0.2193	54

¹ Standard protein ² Low protein ³Means in each column with different superscripts differ statistically ($p<0.05$).

Conclusions supplementation of Fermacto decreased total cholesterol and abdominal fat pad in broilers fed standard protein diet but at low protein diet, just abdominal fat pad was decreased by Fermacto supplementation; therefore inclusion level of Fermacto and level of protein were important for effectiveness. Use of Fermacto was one of the biological ways to improve economic burden in poultry production with decreasing abdominal fat percentage and improve human health by decreasing cholesterol level in serum.

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Ileal crude protein digestibility, caecal crude protein retention and digesta viscosity in broilers fed enzyme supplemented rice husk

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Introduction Enhanced nutrient digestion and absorption in enzyme supplemented broiler diets could be either through reduced digesta viscosity or weakening of the cage effect elicited by soluble or insoluble Non – Starch – Polysaccharides (NSP) on nutrient digestion and absorption. Inhibitory effect of insoluble NSP on endogenous protease reduces efficient digestion of nutrients embedded in cell wall matrix of grains. Increased amount of undigested and unabsorbed protein reaching the caecum implies available nutrient for fermentation and increased coliform forming units of such bacteria as *clostridium* that ferment proteins, peptides and amino acids. The study aimed to assess the effect of partial replacement of maize with Rice Husk (RH) supplemented with Roxazyme G2 G enzyme on ileal crude protein digestibility, caecal crude protein retention and digesta viscosity in broilers.

Materials and methods Four diets were formulated consisting of a Maize – Soya bean meal (M/SBM) control and 3 treatment diets in which maize was partially replaced with 15 (75g/kg of diet), 30 (150g/kg of diet) and 45% (225g/kg of diet) RH. The RH based diets were supplemented with Roxazyme G2 G at the rate of 200g/tonne. Titanium dioxide (TiO₂) was added as an indigestible marker. Free access to experimental diets was provided to 48 Ross unsexed broilers from day 7 to 35 post hatch. The birds were distributed into 48 cages of 1 bird per cage with two cages representing a replicate. A group of 12 cages were then randomly assigned to each of the experimental diets. The birds were reared in a well ventilated temperature controlled house. Digesta was collected from the ileum (2cm posterior to Merkel's diverticulum and 2cm anterior to the ileal – caecal – colonic junction). Digesta collection, collection of digesta liquid fraction and determination of digesta viscosity was done by the method of Steinfeldt *et al* (1998) with the exception of type of viscometer used. A Roto Visco 1 from HAAKE (Germany) was used instead of the Brookfield type. TiO₂ analysis was determined using the method of Brandt and Allam (1987). Ileal crude protein digestibility coefficient was calculated using the formula as given below. The experiment was arranged as complete randomized design of 6 replicates and 2 birds per replicate. All data collected were subjected to polynomial regression procedure in SAS.

$$\text{Ileal crude protein digestibility coefficient} = 1 - \frac{(\text{conc. of TiO}_2 \text{ in feed} \times \text{conc. of nutrient in digesta})}{(\text{conc. of TiO}_2 \text{ in digesta} \times \text{conc. of nutrient in feed})}$$

Results Ileal crude protein digestibility, caecal crude protein retention (unabsorbed protein leaving the ileum less the protein voided in faeces) and digesta viscosity were similar across dietary treatments. Low digesta viscosity (1.71mPa.s) was recorded in broilers fed maize-soya bean meal control diet, with corresponding low caecal crude protein retention (2.55g/kgDM) and a high ileal crude protein digestibility (0.989). A similar trend was observed in broilers fed 15 (2.06mPa.s, 3.62g/kgDM and 0.984), 30 (1.85mPa.s, 2.70g/kgDM and 0.988) and 45% (1.86mPa.s, 3.59g/kgDM and 0.986) enzyme supplemented rice husk-based diets.

Table 1 Enzyme effect on ileal crude protein digestibility, caecal crude protein retention and digesta viscosity in broilers

Parameters	M/SBM	M/SBM+ 75g/kg RH	M/SBM+ 150g/kg RH	M/SBM+ 225g/kg RH	SEM	P value
Ileal CP digestibility	0.989	0.984	0.988	0.986	1.54	0.583
Caecal CP retention (g/kgDM)	2.55	3.62	2.70	3.59	0.40	0.161
Digesta viscosity (mPa.s)	1.710	2.060	1.850	1.860	0.17	0.175

mPa.s: millipascal seconds, CP: crude protein

Conclusion The results show a relationship between ileal crude protein digestibility, caecal crude protein retention and digesta viscosity. The amount of protein reaching the caecum is dependent on the amount absorbed in the ileum which can be influenced by ileal digesta viscosity. The inhibitory effect of high amount of insoluble NSP present in rice husk on endogenous protease could have been minimized by the enzyme utilized via disruption of cell wall matrix.

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Immunomodulatory effects of dietary *Allium cepa* in chicken after immunization

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Introduction Although antibiotics treatment has been used as an efficient technique to control infectious diseases in poultry industry, there are intensive studies to decrease the use of antibiotics because of the increase in microbial resistance. Therefore, enhancing protective immunity with immunostimulators by magnifying the capacity of immune response seems to be the most promising and practical approach. *Alliums* have been reported to possess therapeutic properties often attributed to sulphur-containing compounds (Bonaccorsi *et al.*, 2005). In addition to these compounds, *Allium cepa* is among the richest dietary flavonoids sources; those flavonoids have shown in mammals *in vitro* antimicrobial and anticarcinogenic activities (Block *et al.*, 1992). However, very few studies aimed at examining their effects in poultry have been performed. The objective of this study is to examine if the addition of supplementary *Allium cepa* to a balanced ration enhances the immune response of chickens after immunization.

Materials and methods One-day-old, White Leghorn male chicks were distributed into 3 groups of 8 each. The birds were housed in wire cages, 24 hr light-program and 24°C. After the first week, birds were fed on diets containing powder of *Allium cepa* with 10 or 30 gm/kg of the basal starter diet. Free access to water and feed was allowed. Feed intake and body weight were recorded for growth performance study. On day 14, chickens were immunized intraocularly with Newcastle disease vaccine (NDV-clone 30), and intravenously with *Brucella abortus* (BA), and repeated 14 days later. Blood samples were drawn from wing vein weekly for antibodies determination. Anti-NDV antibody was determined by hemagglutination inhibition (HI) titre whereas anti-BA antibody was determined by agglutination titre. Ratios of CD4⁺ and CD8⁺ T-lymphocytes, and CD4⁺CD8⁺ lymphocytes (mostly B-lymphocytes) in splenocytes were studied. Thus, splenocytes were incubated with FITC-labelled anti-chicken CD4 antibody and PE-labelled anti-chicken CD8 antibody; ratios were determined using flow cytometry. To check *in vitro* co-mitogenic properties of *Allium cepa* extract on B-lymphocyte proliferation, bursocytes were resuspended in RPMI-1640 media to 1×10^6 cell/ml, and B-lymphocytes proliferation was induced by phorbol 12-myristate 13-acetate (PMA). After 48 hr incubation (39.5°C and 5% CO₂), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added, and finally, light absorbance was measured at 570 nm wave length as described by Bao (1998) with some modifications. Proliferation was expressed as stimulation index (SI = proliferation of B-lymphocytes in culture with PMA/proliferation without PMA). Data were analyzed using single factor ANOVA of Microsoft Excel. P<0.05 was considered significant for all analysis.

Results Average feed conversion ratios of *Allium cepa*-fed chickens had values of 2.15 for low concentration and 2.17 for high concentration which were significantly improved (P<0.01, P<0.001, respectively) compared to control (2.70). Feeding low concentration of *Allium cepa* exerted a significant increase in anti-NDV antibody production started in response to primary immunization (Figure 1A). Anti-BA antibody titre was higher with dietary *Allium cepa* in response to secondary immunization; however, low concentration had stronger and prolonged stimulatory effect (Figure 1B). Supplementary high concentration of *Allium cepa* significantly increased the ratio of CD4⁺CD8⁺ cells (48.6%, P<0.001), while decreased that of CD4⁺ (21.0%, P<0.05) compared with control (43.0% and 25.6%, respectively). Adding 1.3 ~ 6.5 µg/ml as a final concentration of *Allium cepa* extract had a higher (P<0.001) SI of 2.52 compared with that of PMA only (1.86).

Figure 1 Effect of dietary *Allium cepa* (L, 10 or H, 30 gm/kg diet) on anti-NDV (A) and anti-BA (B) antibody titres (log₂) in chicken. (*P<0.05, **P<0.01)

Conclusion The results of this study clearly showed that dietary supplementation of White Leghorn-type chicken with *Allium cepa* stimulated humoral immune response and growth performance. As well, *Allium cepa* had *in vitro* co-mitogenic effect on PMA-induced B-lymphocytes proliferation.

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Nutritive value of poultry by-product meal from Iran in broiler feeding

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Introduction Recently, production of poultry by – product meal (PBPM) has been increased in the northwest of Iran, East Azarbijan. PBPM has a profile of available essential amino acids and is rich in calcium, phosphorus and vitamin B12 (NRC 1994). The chemical composition and mineral content can vary greatly depending on raw material and processing conditions. In our country, fish meal is a common ingredient as animal protein in poultry diets. However, fish meal is imported at high cost. In recent years, there have been attention paid to PBPM supplementation in diets by East Azarbijan poultry feed industry. Therefore, the present study was conducted to determine the chemical composition, mineral content and to study the effects of different dietary concentration (3, 6 and 9%) of PBPM on broiler performance.

Materials and methods The three composed PBPM samples were collected from rendering units of industrial poultry slaughter-house in East Azarbijan province and were chemically analyzed according to AOAC (1992) procedures. For feed evaluation 240 Ross-308 as hatched broiler chickens were used in this experiment. Four different treatments were formed in the study. First treatment was control with no PBPM. The other treatments were 3.0, 6.0 and 9.0% PBPM supplementation, respectively. Experimental diets were formulated as isonitrogenous and isocaloric (based on Ross308 management guide book) and fed from 22 to 49 days of age. Broiler chickens were assigned randomly to four treatment groups (per treatment/5 pens). Fifteen broilers were housed on litter-floor. Feed and water was available on an *ad libitum* basis. Broilers were weighed individually at 22, 42 and 49d of age. For each pen, feed consumption ratio (FCR) and weight gain were measured on a weekly basis. Feed intake was weighed back on the same day that body weights (BW) were determined. Mortality was not observed in this study. The data was analyzed in a completely randomized design using GLM procedure of SAS. Comparison of means was conducted by Duncan's multiple range tests.

Results The average of chemical composition and mineral contents of the PBPM samples are shown in Table 1, and the effects of supplementation PBPM to broiler diets on various live performances is shown in Table 2. Feed intake and weight gain in first stage of experiment with increasing inclusion level of poultry by- product meal, were decreased ($p<0.05$). But feed intake and weight gain in second stage of experiment due to adaptation were not significant difference with control birds ($P>0.05$). Weight gain in total experiment period was less than control birds ($p<0.05$). Feed conversion ratio in two stage and total experiment period were similar to standard feed conversion ratio in Ross308 guide book and non significant difference with control bird ($P>0.05$).

Table 1 Composition of PBPM samples (% as fed)

Composition	DM	CP	EE	TVN*	Ca	P	Na	K	Cu	Zn	Mn	Fe
Average	95.5	62.12	25.28	5.6	1.3	0.43	0.61	0.53	0.061	0.173	0.042	0.821

*Total volatile nitrogen

Table 2 The effects of PBPM inclusion in broiler diets at different levels on growth performance

Treatments	22-42d				43- 49 d			22-49d		
	Feed Intake (gr)	Weight Gain (gr)	Body Weight (gr)	FCR	Feed Intake (gr)	Weight Gain (gr)	FCR	Feed Intake (gr)	Weight Gain (gr)	FCR
Control	2398 ^a	1390 ^a	1919 ^a	1.72 ^a	1159 ^a	570 ^a	2.04 ^a	4037 ^a	2281 ^a	1.76 ^a
3%	2278 ^b	1346 ^a	1800 ^b	1.69 ^a	1118 ^a	552 ^a	2.02 ^a	3875 ^a	2201 ^b	1.76 ^a
6%	2170 ^c	1269 ^b	1664 ^c	1.71 ^a	1172 ^a	599 ^a	1.96 ^a	3785 ^b	2134 ^b	1.77 ^a
9%	2066 ^d	1177 ^c	1561 ^d	1.75 ^a	1133 ^a	569 ^a	2.00 ^a	3629 ^c	1984 ^c	1.82 ^a
SEM	28.7	22.2	26.5	0.025	33.2	16.4	0.08	44.2	22.7	0.025

In the same column differently superscripted are significantly ($P<0.05$) different.

Conclusions In summary, the results showed that composition of studied PBPM samples were different from NRC (1994). Also, under the conditions of this study, using of different levels of poultry by product meal (3, 6 and 9 percent) did not show any detrimental effect on feed conversion ratio but depressed broiler growth rate age of 22 to 49 days.

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Effects of addition of Natuzyme® to broiler diets containing different levels of canola meal

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Introduction Canola meal (CM) is a suitable protein source in poultry diets, although it contains a number of antinutritive factors including non-starch polysaccharides and phytic acid. These factors can limit inclusion rate of canola meal in poultry diets (Kocher *et al.*, 2000). The negative correlation between NSP levels and nutritive value of the diet has been demonstrated in poultry (Choct and Annison, 1990). In addition to reducing the availability of phosphorus to birds, phytates are also associated with a number of antinutritional effects, largely because they can chelate divalent cations and reduce protein availability (Ravindran *et al.*, 1995). Successful use of enzymes in cereal-based diets has stimulated interest in the application of enzymes to target the vegetable protein components of poultry diets as well. The current study was, therefore, conducted to investigate the effects of a multi-enzyme on performance and serum thyroid hormone concentrations of broilers fed rations in which soybean meal was replaced by CM.

Materials and methods Eight hundred day-old broiler chicks (Cobb) were randomly allocated to 8 dietary treatments in a 4 x 2 factorial arrangement with 5 replicates per treatment and 20 birds per replicate. Experimental diets had different levels of canola meal (0, 100, 200 and 300 g/kg), with or without enzyme (Natuzyme, Sunnybank, Brisbane, Australia). The supplemental enzyme activities reported by the manufacturer were cellulase 6000 units/g, xylanase 10,000 units/g, amylase 700 units/g, glucanase 700 units/g, phytase 500 units/g, pectinase 70 units/g, proteases 3000 units/g and lipase 30 units/g. The diets were isoenergetic and isonitrogenous and were provided *ad libitum* throughout a 42 d experiment. Feed intake and weight gain were determined and feed conversion ratio was calculated. At 42 d age, 2 birds were randomly selected from each replicate and blood samples were collected for measuring T3 and T4 concentrations. T3 and T4 concentrations in the sera were determined by RIA according to the procedure of Kloss *et al.* (1994). Data were subjected to the GLM procedure for ANOVA (SAS, 2001). Mean separation was accomplished using Duncan's multiple range tests.

Table 1 Effects of canola meal and multi enzyme on performance and serum thyroid hormone levels of broilers

Item		Feed intake(g)	BW gain (g)	Feed:gain (g/g)	Feed intake(g)	BW gain (g)	Feed:gain (g/g)	Serum T3 (µg/ml)	Serum T4 (µg/dl)
		1-21 d			1-42 d				
Enzyme	CM								
No	0	924 ^b	688 ^{ab}	1.34 ^c	3725	2221 ^{ab}	1.68 ^{bc}	1.33	0.63
10		921 ^b	606 ^c	1.52 ^{ab}	3726	2075 ^{bc}	1.79 ^{ab}	1.21	1.36
20		952 ^{ab}	653 ^{abc}	1.46 ^{abc}	3711	2039 ^{bc}	1.82 ^a	1.33	0.4
30		969 ^{ab}	618 ^{bc}	1.57 ^a	3696	1935 ^c	1.91 ^a	1.36	0.83
Yes	0	965 ^{ab}	710 ^a	1.36 ^{ab}	3690	2268 ^a	1.62 ^c	1.37	1.44
10		991 ^a	637 ^{abc}	1.55 ^a	3707	2104 ^{ab}	1.76 ^{abc}	1.41	0.64
20		971 ^{ab}	640 ^{abc}	1.51 ^{abc}	3705	2103 ^b	1.75 ^{abc}	1.25	0.48
30		956 ^{ab}	643 ^{abc}	1.48 ^{abc}	3681	2087 ^{bc}	1.76 ^{abc}	1.24	0.66
S.O.V									
Enzyme	*	ns	ns	ns	*	*	*	ns	ns
CM	ns	**	*	ns	**	*	*	ns	ns
Enzyme × CM	*	*	*	ns	*	*	*	ns	ns
SEM		14.6	16.2	0.02	37.1	35.3	0.03	0.06	0.23

^{a-c}Means with different superscripts are significantly different. ^{ns}non-significant *P<0.05 **P<0.01

Results Canola meal had no effect on feed intake. Body weight gain was significantly reduced when canola meal was added into the diets (P<0.01). No effect of enzyme addition was observed on BW gain and feed:gain during 1 to 21 d. Enzyme addition significantly increased body weight gain during 1-42 d (P<0.05). Feed conversion ratio was highest from 1 to 42 days when 30 g/kg canola meal was added to the diets. Enzyme supplementation significantly (P<0.05) improved feed conversion ratio during 1- 42 d. The interactions between enzyme and canola meal for body weight gain and FCR were significant. The effect of enzyme addition on weight gain and feed:gain was numerically greater in diets which had higher levels of CM. Different levels of added canola meal and enzyme had no significant effect on concentrations of serum thyroid hormones.

Conclusions The results of this study showed that inclusion of CM at or above 100 g/kg depressed weight gain and FCR without affecting the feed intake. Enzyme supplementation had the potential to reduce the unfavourable effects of canola meal.

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The effects of multi-enzyme addition on performance of broiler chicks

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Introduction Today the use of enzymes is common in practical poultry nutrition. Enzyme supplementation usually result in numerous beneficial effects, such as increased utilization of nutrients (e.g. fat & protein), improved AME values, increased growth rate, improved feed conversion ratio, decreased viscosity of intestinal digesta, reduced incidence of sticky excreta and improved litter conditions (Broz and Ward., 2007). The use of multi enzymes to improve the digestibility of corn-soybean meal-wheat diets for broilers is less well documented than wheat and barely diets. Therefore, the aim of the present study was to examine the effects of a multi-enzyme supplementation on the performance of broiler chicks fed on corn-soybean meal-wheat diets.

Materials and methods A total of 150 one-day-old mixed-sex broiler chicks (Cobb 500) were used in a completely randomized design with 3 replicates with 25 chicks in each replicate. The birds were randomly allocated to 6 pens. The main ingredients of diets included corn, soybean meal and wheat. The amount of corn, soybean meal and wheat in starter (1-10 d) and grower (11-28 d) diets were about 437, 356, 150 and 374, 314, 250 g/Kg diet respectively. Diets were formulated according to Cobb 500 rearing guideline and contained 2950 Kcal/Kg metabolizable energy and 22% crude protein during starter, and 3000 Kcal/Kg metabolizable energy and 21% crude protein during grower period. Feed and water were provided *ad libitum* during the experiment. Temperature was maintained at 32° C for the initial 3 d and then gradually reduced according to normal management practices, until a temperature of 22° C was achieved. The experiment lasted 4 weeks. The experimental diets contained 2 levels of a dietary NSP degrading enzyme (0, 0.05%; Endofeed W produced from *Aspergillus niger*, with minimum activity of 2250 u/g xylanase and 700 u/g Beta-glucanase). According to the manufacturer, the allowance for this enzyme product was 0.05% and it also contained activities of other enzymes, including cellulase, protease, α -amylase and α -galactosidase. The enzyme complex was added to the chicken diet as a dry powder from hatching to the end of the experiment. Chickens were weighed weekly (from 1 to 28 d) to determine their performance. Data were analyzed by analysis of variance using the GLM procedure (SAS institute, 2001). Differences among means were compared by Duncan's multiple range test (1955).

Results Data obtained from this experiment indicated that enzyme supplementation significantly improved body weight gain and feed conversion ratio (Table 1). These results are in agreement with the findings of previous studies (Wang *et al.*, 2005; Gao *et al.*, 2007). Adding enzyme to broiler diets may improve performance by two mechanisms: increasing feed intake and improving nutrient digestibility. Both mechanisms might be induced, at least partially, by reduction of gut viscosity which decreased retention time of digesta in the gut, allowing more feed consumption and therefore improving growth and feed conversion ratio (Lázaro *et al.*, 2003). The results showed that enzyme supplementation to corn-soybean meal-wheat based diet can improve weight gain whilst maintaining intake, which is suggestive of improved digestibility of a limiting nutrient.

Table 1 Effect of multi-enzyme supplementation on broilers performance during 1 to 28 d of age

Diets	Feed intake (g)	Weight gain (g)	FCR (g:g)	Energy efficiency ratio ¹ (%)	Protein efficiency ratio ² (g:g)	Growth ratio ³
1 (without enzyme)	2094.44	1389.95 ^b	1.51 ^a	22.10 ^b	3.142 ^b	34.74 ^b
2 (with enzyme)	2095.05	1435.68 ^a	1.46 ^b	22.80 ^a	3.245 ^a	35.89 ^a
s.e.m	13.05	7.13	0.008	0.13	0.016	0.18

^{ab} Means with different superscripts in each column are significantly different ($P < 0.05$)

¹Calculated as weight gain $\times$ 100 divided by total Kcal ME intake. ²Calculated as weight gain divided by protein intake.

³Calculated as BW at 28 d of age – BW at 1 d of age / BW at 1 d of age.

Conclusions Results of the present study indicated that supplementation of enzyme at the level of 50 mg/Kg diet significantly improved feed conversion, energy and protein efficiency and growth ratio of Cobb 500 broiler chicks during starter and grower period of rearing (1 to 28 d of age).

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Enrichment with long chain omega-3 fatty acids and sensory evaluation of chicken meat

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Introduction N-3 fatty acids are essential for normal growth and development, and may play an important role in prevention of coronary artery disease, hypertension, diabetes, arthritis, other inflammatory and autoimmune disorders and cancer in humans (Simopoulos, 1999). Fatty acid profiles of broiler meat may be modified by adding fish oils to the diet (Lopez-Ferrer *et al.*, 2001). When meat is enriched with PUFA, particularly n-3 long-chain fatty acids ($C \geq 20$), all sources of added vegetable oils seem to be less effective than marine oils (Bou. R *et al.*, 2004). The purpose of this experiment was to study the effect of dietary fish oil on fatty acid composition of thigh and breast meat in broiler chickens.

Materials and methods Four hundred and fifty d-old Ross 308 male, broiler chickens were allocated to 6 dietary treatment with 5 replicates of 15 birds each. The birds were reared in a controlled environment house and had *ad libitum* access to water and feed. All dietary nutrients were provided as to meet AVIAGEN recommendations. Khazar Kilka fish oil was added at the levels of 0, 10, 20, 30, 40 and 50 g/kg in diets, fed from 28 to 42d of age. Performance criteria were measured during the experimental period. One bird with an average live body weight of each replicate group was selected and slaughtered on day 42 d The left thigh and left side of the breast were excised and stored at -20°C for later analysis. Samples (including skin) were analyzed for n-3 fatty acids with GC and sensory evaluation after each sample was individually cooked in boiling water. Data were analysed by using GLM procedure of SAS (9.1)

Results Weight gain and Feed Conversion Ratios were similar ($P > 0.05$) for all treatments (table1). The Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) contents of thigh or breast tissues were increased significantly ($P < 0.05$) as the level of dietary fish oil increased. Effects were not significant for LA(linoleic acid) and ALA(alpha linolenic acid). Sensory evaluation showed that panellists did not identify the fishy smell of cooked thigh or breast meat of birds fed diets with 0, 10 or 20 g/kg fish oil. The concentration of DHA in breast and thigh meat increased from 0.046 (mg/g) and 0.086 (mg/g) to 0.166 (mg/g) and 0.27 (mg/g), respectively, when dietary fish oil increased from 0 to 20 g/kg,. Significant linear relationships were found between the levels of fish oil and EPA in the breast and thigh ($R^2 = 0.59$; $R^2 = 0.71$ respectively) and between the fish oil and DHA in the breast and thigh ($R^2 = 0.71$; $R^2 = 0.72$, respectively).

Table 1 Broiler chickens performance and concentration of some n-3 FAs in breast and thigh meat (mg/g in meat) of birds given diets including fish oil

Treatments	WG (g/b/d)	FCR	Thigh				Breast				Scores ¹	
			C18:2	C18:3	C20:5	C22:6	C18:2	C18:3	C20:5	C22:6	Thigh	Breast
control	69.8	2.24	5.4	0.35	0.028 ^b	0.086 ^c	0.54 ^b	0.02 ^b	0.014 ^b	0.046 ^c	1.10	1.12
1% fish oil	71.4	2.27	6.2	0.13	0.051 ^b	0.16 ^{bc}	0.87 ^{ab}	0.04 ^{ab}	0.042 ^b	0.145 ^{bc}	1.27	1.17
2% fish oil	69.2	2.48	6.1	0.42	0.086 ^{ab}	0.27 ^{bc}	0.99 ^{ab}	0.10 ^a	0.04 ^b	0.166 ^{bc}	1.25	1.25
3% fish oil	72.1	2.29	4.6	0.32	0.16 ^{ab}	0.38 ^{ab}	0.67 ^{ab}	0.03 ^{ab}	0.04 ^b	0.240 ^{ab}	2.32	2.25
4% fish oil	67.6	2.53	4.2	0.43	0.23 ^a	0.53 ^a	0.99 ^{ab}	0.07 ^{ab}	0.09 ^a	0.340 ^a	3.25	3.87
5% fish oil	68.0	2.44	3.2	0.30	0.16 ^{ab}	0.58 ^a	1.10 ^a	0.06 ^{ab}	0.08 ^a	0.290 ^a	4.02	4.25
SEM	1.78	0.04	0.09	0.11	0.05	0.08	0.16	0.04	0.01	0.04		
P value	NS	NS	NS	NS	0.003	0.003	NS	NS	0.006	0.001		

^{abc} Values in column with no common superscripts differs significantly ($P < 0.05$)

¹ Flavour scores using a 5 point scale: 5=very poor, 4=poor, 3=indifferent, 2=acceptable, 1=typical chicken flavour

Conclusion The levels of long chain fatty acids (EPA and DHA) were linearly increased in the chicken meat as the levels of dietary Khazar Kilka fish oil increased from 0 to 5%. The panellists identified the fishy smell of the meat when more than 20 g/kg fish oil was included in the diet.

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Determination of chemical composition and metabolizable energy of wastes of spaghetti, pasta, biscuit, crisp, chickpea pre-cleaning and chickpea screening plants

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Introduction Based on the official statistics of industries and mines organization about 1000 ton wastes of spaghetti, pasta, biscuit, crisp, and about 7500 ton waste of chick pea pre-cleaning plants are produced annually in East Azerbaijan province of Iran. In our country there is little researches on wastes. In one study, ileal amino- acid digestibility of wheat, autoclaved wheat and spaghetti by-products for broiler chicks was determined (Zaghari 2006). The aim of the present study was to determine metabolizable energy and chemical composition of wastes of spaghetti, pasta, biscuit, crisp, chickpea pre-cleaning and chickpea screening plants.

Materials and methods After classified random sampling from 10% of spaghetti, pasta, biscuit, crisp, chickpea pre-cleaning and chickpea screening plants, the samples were ground and mixed. 24 adult laying-type cockerels (Hy-Line W36, 35-week-old) with mean body weight of 2000 g $\pm$ 100 were randomly grouped into six groups of four replicates. All birds were kept in individual battery cages (25 cm $\times$ 35 cm $\times$ 50 cm in dimension) and fed commercial diets prior to the experiment. The wastes of spaghetti, soup pasta, rice pasta, crisp, biscuit, pre-cleaning chickpea, and chickpea screening were mixed in the ratio of 15% to basal diet. Then, in order to determine metabolizable energy (TME, TMEn) of waste, 30 grams of mixed feed were force-fed to 4 adult Leghorn-type roosters, according to the method described by Sibbald (1986). Excreta voided from each bird following the feeding procedure was collected quantitatively, for 48 h. Birds had free access to water, and when not on experiment, to a commercial diet. All the birds remained healthy and survived the experimental procedure. The chemical analysis of wastes and collected samples of excreta was carried out according to the standard methods of analysis (AOAC,1990). Gross energy of wastes and individual samples of excreta was measured by a bomb calorimetry.

Results Summarized in table1, are data showing chemical composition of studied wastes. Maximal crude protein, ash and crude fiber value was obtained with chickpea pre-cleaning.

Table 1 Chemical composition (g/kg fresh weight) of wastes

wastes	Dry Matter	Ash	Crude Protein	Crude Fat	Crude Fiber	NFE	NDF	ADF	Gross Energy (MJ/kg)
spaghetti	915	12	127	24	2	805	17	1	18.79
pasta	909	7	141	65	2	755	14	3	18.74
biscuit	948	20	90	170	51	649	111	17	20.37
crisp	935	15	92	42	5	826	131	3	18.23
chickpea pre-cleaning	923	73	302	87	178	320	323	224	19.38
chickpea screening	919	60	300	78	78	450	351	96	19.79

Table 2 Metabolizable energy of experimental wastes (MJ/kg)

wastes	AME	AMEn	TME	TMEn
spaghetti	14.41 ^d $\pm$ 0.17	14.8 ^c $\pm$ 0.18	15.77 ^d $\pm$ 0.17	15.23 ^c $\pm$ 0.18
pasta	15.69 ^c $\pm$ 0.19	16.24 ^b $\pm$ 0.25	17.06 ^c $\pm$ 0.19	16.67 ^b $\pm$ 0.25
biscuit	16.26 ^a $\pm$ 0.09	16.78 ^a $\pm$ 0.26	17.26 ^a $\pm$ 0.09	17.22 ^a $\pm$ 0.22
crisp	15.98 ^b $\pm$ 0.13	16.25 ^b $\pm$ 0.20	17.34 ^b $\pm$ 0.13	16.69 ^b $\pm$ 0.20
chickpea pre-cleaning	10.89 ^e $\pm$ 0.13	10.34 ^f $\pm$ 0.16	10.96 ^f $\pm$ 0.22	11.64 ^d $\pm$ 0.17
chickpea screening	9.6 ^f $\pm$ 0.22	10.34 ^f $\pm$ 0.16	10.96 ^f $\pm$ 0.22	10.77 ^f $\pm$ 0.16
SEM	0.088	0.098	0.088	0.098

Among the wastes, biscuit had the highest ME, presumably related to its highest fat content. Statistical analysis showed that there were significant differences ($p < 0.05$) between ME types of wastes.

Conclusions These results show that experimental wastes especially chickpea, biscuit and spaghetti are rich sources of protein and energy with some values even exceeding those in corn and wheat. Further work is required to test their suitability in diets for poultry.

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The effect of different lightning programs on reproductive performance of native turkeys

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Introduction Lighting is very important for turkey production, because their life period is longer than poultry (Nixey 1994). One of the important effects of lighting is to change the time of sexual maturity in pullets. Classen *et al.* (1994) concluded that constant light causes an increase incidence of leg problem and metabolic disorders. With lighting programs and lighting intensity, time of sexual maturation can be modified. Because there is little information about lighting programs in native turkey production in Iran, the aim of this research is determining the best lighting program for native turkey production.

Materials and methods The effect of 2 lighting program in growth period and 7 lighting program in production period on native turkey's reproductive performance was surveyed. Turkeys received (7L:17D) and (14L:10D) lighting programs by 36 weeks respectively. 400 female and 80 male turkeys were used on completely randomized design with four repeat, each contain 50 female and 10 male turkeys. The survey of lighting program continued in production period (37- 66 weeks). The groups receiving 7h light during the growth period received 4 different lighting program (12L:12D), (14L:10D), (16L:8D) and intermittent (1L: 3d) for 66 weeks. The group receiving 14h light during the growth period received 3 different lighting programs (14L:10D), (16L:8D) and (1L:3D). This period of experiment was carried on completely randomized design with four repeat, each contain 10 female and 2 male turkeys. Sexual maturity age, egg production, fertility, and hatchability percent and feed intake for every 1day's chick production were recorded.

Results The largest hatchability was in 7L: 17D in growing period and then 14L:10D in production period group, that was significant differences with 14L:10D in growing period and (1L:3D) in production period group ($p<0.05$). The least feed intake for every 1 day's chick production was in 14L:10D in growing and production period group, but there wasn't significant differences between treatments ($p<0.05$). The largest produced chick was in 7 and 14 lights respectively for growth and production period group, but there wasn't significant differences between treatments ($p<0.05$).

Table 1 Number of chicks produced from 100 female turkey, amount of feed intake(kg) for every 1day old chick production and mean of maturity age

Lighting program		Number chicks produced from 100 female turkey in every day	Fertility	Hatchability	Feed intake(kg) for chicken production	FI(kg) every chick production	for 1d	Mean of maturity age(day)
Growth period	Production period							
7L:17D	1L:3D ¹	7.05	64 ^c ±20	77 ^{ab} ±18	54.93	7.79		
7L:17D	12L:12D	9.30	72 ^b ±19	80 ^{ab} ±14	30.29	3.27		259.25
7L:17D	14L:10D	11.94	84 ^a ±12	85 ^a ±21	28.32	2.37		
7L:17D	16L:8D	10.55	77 ^{ab} ±12	79 ^{ab} ±19	42.52	4.03		
14L:10D	1L:3D	8.19	72 ^{bc} ±26	71 ^b ±15	22.45	2.74		
14L:10D	14L:10D	10.18	62 ^{bc} ±19	85 ^a ±15	22.48	2.21		256.67
14L:10D	16L:8D	10.80	78 ^{ab} ±17	81 ^{ab} ±17	28.94	2.68		

1-1L:3D=Intermittent program (1h light:3h dark). 2-7L:17D=continuous program(7h lightness:17h dark)

Conclusion The best FCR was for the groups received 14h L in growth period and 14h D in production period and also in the total production period, the highest egg production percent was for the groups received 7h L in growth period and 14h L in production period

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Effects of butyric acid, mannanoligosaccharide (MOS) and avilamycin on performance and small intestine morphology of broiler chickens

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Introduction The subtherapeutic use of antibiotics in animals has been under scientific and public scrutiny as antibiotic growth promoters (AGP) have been linked to the development of antibiotic resistance in bacteria, which poses a threat to human health (Smith *et al.*, 2003). Short chain fatty acids such as butyrate are considered as potential alternative to AGP. In addition to its bactericidal activity; butyrate appears to play a role in development of the intestinal epithelium (Leeson 2005). Prebiotics (e.g. mannanoligosaccharides, MOS) are nondigestible feed ingredients that can selectively stimulate growth or metabolic activity of a limited number of intestinal microorganisms (Gibson and Roberfroid, 1995). This study was, therefore, conducted to investigate effectiveness of mannanoligosaccharides and butyric acid as potential alternatives to AGP in broilers.

Materials and methods Two hundred and eighty eight one-day-old male broiler chicks (Arbor Acres Plus) were raised over a 42 d experimental period. Dietary treatments were: 1) Negative control, antibiotic free diet; 2) Positive control diet (containing 15mg/kg of avilamycin); 3) Negative control with MOS (Bio-Mos, Alltech Inc., Nicholasville, KY, USA, 2g/kg of the diet) and 4) Negative control with butyric acid (BabyC4, SILO, Industria Zootecnica, Florence, Italy, 3 g/kg of the diet to 21 days). Chicks were randomly assigned to 4 replicates per treatment and 18 birds per replicate. Dietary treatments were fed during starter (0-14 d), grower (14-28 d) and finisher (29- 42 d) periods. All diets within each period were prepared with the same batch of ingredients and had the same nutrient composition. For intestinal morphometric examination, two birds per replicate (8 birds per treatment) were euthanized at day 28 and 2-cm segments of the midpoint of the jejunum were removed and fixed in 10% buffered formalin. Serial sections were cut at 5 µm and placed on glass slides. Sections were deparaffinized in xylene, rehydrated in a graded alcohol series, stained with hematoxylin and eosin, and examined by light microscopy. Data were analyzed using the GLM procedure of SAS (2001). Differences among treatments were compared using a Duncan's multiple range tests.

Results The effects of addition of avilamycin, butyric acid and MOS on broiler performance, feed intake, body weight, villi height and crypt depth in the jejunum are shown in table 1. Birds fed MOS and butyric acid were significantly heavier than negative controls ($P<0.05$). There were no significant differences among dietary additive groups with respect to body weight gain. Dietary supplementation with avilamycine, butyric acid and MOS significantly improved FCR ($P<0.01$) although this improvement was greater in butyric acid and MOS compared to avilamycin groups. Carcass yield in butyric acid groups was greater than negative controls ($P=0.08$). Birds fed avilamycin had lowest small intestine weight ($P<0.01$). There were no significant effects of feed additives on villi height and crypt depth.

Table 1 Effects of avilamycin, butyric acid and MOS on growth performance, villi height, crypt depth, small intestine weight and carcass yield of broiler chickens

Treatment	BW gain 1-42days (g)	FCR 1-42days	Villi Height (µm)	Crypt depth (µm)	Small Intestine (% of BW)	Carcass yield (% of BW)	Breast weight (% of BW)
Negative control	2212 ^b	1.99 ^a	862	201	4.56 ^a	71.9	27.1 ^b
Avilamycin	2344 ^{ab}	1.83 ^b	871	189	3.45 ^c	74.5	29.8 ^a
Butyric acid	2370 ^a	1.72 ^c	925	194	3.85 ^{cb}	75.3	30.3 ^a
MOS	2376 ^a	1.75 ^c	869	209	4.14 ^{ab}	72.8	29.0 ^a
SEM	48.8	0.03	46.3	9.6	0.152	0.95	0.51
Significance	*	**	NS	NS	**	NS	**

^{a-c} Means in a column without a common superscript are significantly different. ^{NS} non-significant * $P<0.05$ ** $P<0.01$

Conclusions It was concluded that addition of butyric acid and MOS can improve broiler performance, without significant effects on intestinal morphology, including villus height and crypt depth. No difference in performance was observed between the two additives. The inclusion of avilamycin as a growth promoter was also associated with positive effects on performance of broilers. The results of this study indicated that addition of butyric acid and MOS to the diet could be an alternative to the use of antibiotics as growth promoters in broiler production.

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Growth curve models for commercial pullets under severe heat stress condition

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Introduction The objectives of our study were to propose a growth curve and to develop a mathematical model to describe the body weight of pullet experiencing severe heat stress (42°C). Poultry producers who raise their own replacement pullets can control their pullet's growth, condition and development. Many of the problems which occur during the early part of lay can be traced back to insufficient or improper type of body weight attained during the various stages of the growing period. In order to avoid these problems, the body weight of pullets must be controlled. Rearing conditions for pullets vary depending on environmental pressures and can affect growth rate. Without the basic knowledge of the flock grow-out, it is virtually impossible to understand and possibly solve problems which may later occur during the laying period. It must be kept in mind that once egg production begins, it is too late to solve problems resulting from growing period. The two most important criteria of pullet quality are uniformity within the flock and proper body weight at a specific age. Almost anything that adversely affects a pullet will usually be reflected in lower body weights and poorer flock uniformity. High ambient temperatures can be devastating to commercial pullet growth rate; coupled with high humidity they can have an even more harmful effect on proper and recommended body weight. Heat stress interferes with the poultry comfort and suppresses performance efficiency. In order to verify the effect of heat stress on pullet growth rate, many curve modelled, fitted and verified to proposed best one.

Materials and methods 2250 leghorns (HyLine-W36) pullets were used for the study which was conducted during the hot months of year in Mollasani educational and research centre (Ramin agricultural university, north west of Iran). All the bird fed isoenergetic and isonitrogenous diets. Body weight, age, mortality and DMI were recorded every week. Temperature and humidity were recorded and the Thermal-Humidity Index (THI) was calculated every day. The collected data were analysed by the Statistical Analysis Systems (SAS) NLIN procedure (SAS, 2005), and CurveExpert 1.3 software to fit and compare the Gompertz, Richards, Weibull, MMF, Logistic, Von bertalanffy and some other curves.

Results The most fitted one was Gompertz model with $r=0.998$ and $MSE=24.11$ and with parameters $a=1441.431$; $b=1.205$; and $c=0.023$. Consequently, under heat stress condition the growth model proposed by company is changed, and this must be considered by producers to achieve the best profit in production period.

Table 1 comparing some models fitted for pullet reared in hot environment conditions.

Model Name	Coefficient of determination	Standard Error	Parameter of model			
			A	b	c	d
Gompertz	0.998423	24.107	1441.4318	1.2053797	0.022682751	
4th Degree Polynomial	0.998499	25.041	50.305531	1.9294874	0.23985574	-0.002306
Richards	0.998352	25.397	1405.1059	-0.7664503	0.024911281	0.11889103
Weibull	0.998344	25.462	1451.4692	1400.4615	0.000732897	1.6011592
MMF	0.998323	25.620	58.263176	3332.0639	1869.7764	1.7846243
Logistic	0.996937	33.583	1265.264	13.149497	0.041046892	

The form of equations mentioned above was as $y=a*\exp(-\exp(b-cx))$ for Gompertz model, $y=a+bx+cx^2+dx^3...$ for 4th Degree Polynomial model, $y=a/(1+\exp(b-cx)^{(1/d)})$ for Richards model, $y=a-b*\exp(-c*x^d)$ for Weibull model, $y=(a*b+c*x^d)/(b+x^d)$ for MMF model and $y=a/(1+b*\exp(-cx))$ for Logistic model. The Coefficient of determination or R^2 was high for all models but the lowest standard error was seen in Gompertz model. So the Gompertz model proposed for its lower standard error and for using 3 parameters.

Conclusion Under heat stress condition the growth model proposed by the company is changed, and this must be considered by producers to achieve the best profit in production period.

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The effect of increasing levels of fish oil on immune responses of broiler chickens

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Introduction There has been interest in the enrichment of poultry meat with long chain n-3 polyunsaturated fatty acids (PUFA) as a means of increasing the low consumption of these acids by humans consuming western diets. There is some concern, however, that at high levels of consumption, n-3 PUFA may have detrimental effects on immune function. However, research to date shows that strong controversy surrounds this immunomodulation. The aim of this experiment was to determine the effects of dietary long chain PUFA on aspects of immune function in broiler chickens.

Materials and methods A total of 18 one-day-old male Ross 308 broiler chicks were reared as a single group for 21 d, and fed a common starter diet. At 21 d, birds were randomly allocated to one of six pens (106x106x108cm), three chicks per pen. Water and feed were provided ad libitum. The broilers were fed for 33 d one of three wheat-soyabean meal based diets. All diets contained 60g/kg added oil, which was either 0,60; 30,30; or 60,0 g fish oil (FO) and soya oil respectively. Chickens were sacrificed between 54 and 60 d of age and samples of blood, bursa of Fabricius, spleen and thymus were collected. The bursa of Fabricius, spleen and thymus were weighed. Heparinised blood was layered on equal volumes of histopaque for preparation of peripheral blood leukocytes. Lymphocyte subsets from the freshly harvested spleen and thymus were prepared and stained with four monoclonal antibodies (mAb): anti-CD3, anti-CD4, anti-CD8 and BU-1A (B cell marker). Immune cells were then enumerated using the FACSCalibur™ flow cytometer. Quantitative analysis of the phagocytic activity of peripheral mononuclear phagocytes in whole blood was performed using phagotest commercial kits, (ORPEGEN-Pharma). Results were expressed as percentage of fluorescent cells and data were analysed by CellQuest™ software. The overall differences of the effects between the three dietary treatments were analysed using one-way analysis of variance (ANOVA) and the general linear model procedure of Minitab was applied in all the tests. Differences between the treatment groups were considered statistically different at $P \leq 0.05$.

Results Increasing levels of fish oil did not affect the weights of the spleen. However, chickens fed diet with 60 g/kg FO had significantly lower bursa weights ($p < 0.01$) than those fed diets with 0 g/kg and 30 g/kg FO (Table 1). Chickens fed diets containing 30 g/kg FO had significantly higher thymus weights compared to chickens fed 0 and 60 g/kg ($p < 0.05$). There was no significant effect of increasing fish oil level on the percentage positive and mean fluorescence intensity (MFI) of the leukocyte subsets in peripheral blood, spleen and thymus. However, the proportion of B-cells in peripheral blood and the MFI of CD8 subsets in the spleen approached significance, $P = 0.058$ and 0.054 , respectively (data not shown). Results of phagocytic activity show that the different levels of FO in the experimental groups neither affected the positive percentage of cells nor MFI. However, there was a trend towards a lower proportion of monocytes being engaged in phagocytosis when broilers were fed diets containing 60 g/kg FO ($p = 0.055$) (Table 2).

Table 1 % Body weight of different tissues

Diet (g/kg FO)	Tissue ¹		
	Spleen	Thymus	Bursa
0	0.12	0.15 ^a	0.30 ^a
30	0.15	0.17 ^b	0.28 ^a
60	0.12	0.12 ^a	0.14 ^b
SE Mean	0.01	0.01	0.02
P value	0.164	0.022	0.001

¹For each tissue weight %, values with different superscripts are significantly different

Table 2 Effect of diet on % of phagocytic leukocytes and their MFI

Diet (g/kg FO)	Monocytes		Granulocytes	
	% of +ve cells	MFI	% of +ve cells	MFI
0	46.78	2148	69.77	1737
30	48.76	2306	82.34	3448
60	35.16	2044	72.84	2204
SE Mean	3.788	630	3.76	992
P value	0.055	0.962	0.102	0.267

Conclusions These results show no evidence of detrimental effect of enrichment with fish oil under the circumstances of this study. However, further studies should be conducted on different immune functions under different situations (e.g. challenge with pathogen), especially as there is an evidence of a potential threshold effect in some cases. Such studies would help the poultry industry to improve or maintain the health status of poultry at an optimum level in circumstances when poultry meat is being enriched with long chain n-3 polyunsaturated fatty acids.

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Effect of dietary Phytase and NSP-degrading enzymes in diets containing rape seed meal on broiler performance

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Introduction In comparison to 44% crude protein of soybean meal (SBM), the protein content of rapeseed meal (RSM) is about 35- 40% and has a physiologically suitable amino acid combination in animal nutrition, but RSM contains nutritionally unfavourable substances such as glucosinolates, sinapin, tannin, phytate and non starch polysaccharides (NSP) (Kocher *et al.*, 2000). Enzymes have the potential to be used in diets contain antinutritional factors that hinder nutrient availability. NSPs include cellulose, B-glucans, arabinoxylans, and pectins that may increase viscosity of digesta and cause a decrease in nutrient digestibility and performance of broiler chickens. Phytase activity from digestive secretions, some feed ingredients, resident bacteria, exogenous microorganisms, or both resident bacteria and exogenous microorganisms is present in the digestive tract of broiler chickens (Kornegay, 2001), but its efficiency at a practical level is very low. It is accepted that broilers lack sufficient levels of phytase activity to effectively hydrolyse the phytate molecule. Phytate-bound P is not well digested, so inorganic P is added to broiler diets that increased feed costs (Lescoat *et al.*, 2005). The purpose of this study was to investigate the replacement value of SBM with locally grown RSM and two types of enzymes (NSP-degrading and phytase) on performance of broiler chickens.

Materials and methods Three levels of (0.0, 25.0 and 50%) SBM protein was replaced with RSM protein and two levels of Phytase enzyme (0 and 500 FTU Phyzyme/kg, Phyzyme XP is a bacterial phytase from *Schizosaccharomyces*), two levels of a dietary NSP-degrading enzyme (0, 0.17%, Grindazyme, minimum activity of 36000 U/g xylanase and 15000 U/g Beta-glucanase) were added to the diets during starter (7-21 days of age) and grower (21-42 days of age) periods. All diets had 0.5% available P and 0.95% Ca, and were isocaloric and isonitrogenous (2969 kcal/kg ME and 21.70% CP in starter; 3118.43 kcal/kg ME and 19.65% CP in grower period). 360 Ross strain chickens were used in a 2×2×3 factorial arrangement in a completely randomized design with twelve treatments, three replicates and ten birds per replicate. Feed consumption and body weight gain of chicks were recorded 4 h after the removal of feed and feed conversion ratio (FCR) calculated at end of every week. All Data were analysed by SAS (2000). Means compared by Duncan's test.

Results The results of this experiment indicated that FI, BWG and feed efficiency of broiler were significantly ($P<0.05$) decreased by increasing RSM in all period of experiment (table 1). These factors were highest in diet without RSM. The BWG and FI of broiler were significantly ($P<0.05$) increased by addition of Grindazyme, but were not affected by supplementation of Phytase in the diet. However Broiler fed diets contains phytase have numerically more FI and BWG than control diet.

Table 1 The main effects of diets contain Phytase and NSP-degrading enzyme and rapeseed meal on broiler performance

	Weigh gain (kg)			Feed intake (kg)			FCR		
	7-21	22-42	7-42	7-21	22-42	7-42	7-21	22-42	7-42
Phytase	0.50	1.31	1.81 ^a	0.79	2.60	3.39 ^a	1.56	1.99	1.87 ^a
No Phytase	0.52	1.33	1.84 ^a	0.78	2.63	3.41 ^a	1.51	1.99	1.85 ^a
SEM	0.01	0.01	0.02	0.007	0.02	0.01	0.03	0.01	0.01
Grindazyme	0.51	1.28 ^b	1.80 ^b	0.78	2.60 ^b	3.37 ^b	1.52	2.02 ^a	1.87 ^a
No Grindazyme	0.51	1.35 ^a	1.86 ^a	0.79	2.64 ^a	3.43 ^a	1.55	1.95 ^a	1.85 ^a
SEM	0.01	0.05	0.04	0.007	0.03	0.04	0.02	0.05	0.01
0% RSM	0.54 ^a	1.36 ^a	1.90 ^a	0.79 ^a	2.65 ^a	3.44 ^a	1.47 ^b	1.95 ^a	1.81 ^b
25% RSM	0.49 ^b	1.31 ^{ab}	1.80 ^b	0.78 ^b	2.62 ^{ab}	3.39 ^{ab}	1.58 ^a	2.00 ^a	1.89 ^a
50% RSM	0.51 ^b	1.28 ^b	1.78 ^b	0.79 ^{ab}	2.57 ^b	3.36 ^b	1.55 ^a	2.02 ^a	1.89 ^a
SEM	0.02	0.04	0.06	0.006	0.04	0.04	0.07	0.04	0.07

SEM: standard error of means; FCR: Feed conversion ratio; RSM: Rape seed meal

Conclusion Levels of RSM showed more adverse effects on performance. It seems addition of enzyme was an effective method to overcome the anti nutrition factor of RSM. Therefore, it may be concluded that NSP-degrading and Phytase enzymes incorporated in rapeseed meal based broiler diet could be beneficial.

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Effect of dietary Phytase and NSP-degrading enzymes in diets containing rape seed meal on broiler carcass characteristics

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Introduction In comparison to the 44% crude protein of soybean meal (SBM), the protein content of rapeseed meal (RSM) is about 35- 40% and has a physiologically suitable amino acid profile for animal nutrition. However, RSM contains nutritionally unfavourable substances such as glucosinolates, sinapin, tannin, phytate and non starch polysaccharides (Kocher *et al.*, 2000). Enzymes have potential to improve the nutrients availability of diets containing antinutritional factorst. NSPs include cellulose, B-glucans, arabinoxylans, and pectins that may increase viscosity of digesta and cause a decrease in nutrient digestibility and performance of broiler chickens. Phytase from digestive secretions, some feed ingredients, resident bacteria and exogenous microorganisms is present in the digestive tract of broiler chickens (Kornegay, 2001), but its efficiency at a practical level is very low. It accepted that broilers lack sufficient levels of phytase activity to effectively hydrolyse the phytate molecule. Phytate-bound P is not well digested, so inorganic P is added to broiler diets that increases feed costs (Lescoat *et al.*, 2005). The purpose of this study was to investigate the replacement value of SBM with locally grown RSM and two enzymes (NSP-degrading and phytase) on the weight of carcass, some organs, and amount of blood-Ca and P of broiler chickens.

Materials and methods Three levels of (0.0, 25.0 and 50%) SBM protein was replaced with RSM protein and two levels of phytase (0 and 500 FTU Phyzyme/kg, Phyzyme XP is a bacterial phytase expressed in *Schizosacchchromyces pombe*), two levels of a dietary NSP-degrading enzyme (0, 0.17%, Grindazyme, minimum activity of 36000 U/g xylanase and 15000 U/g Beta- glucanase) were added to the diets during starter (7-21 days of age) and grower (21-42 days of age) periods of broiler chickens. Enzymes were added to the SBM and RSM diets over the top. All diets had 0.5% available P and 0.95% Ca, and were isocaloric and isonitrogenous (2969 kcal/kg ME and 21.70% CP in starter; 3118.43 kcal/kg ME and 19.65% CP in grower period). 360 Ross strain chickens were used in a 2×2×3 factorial arrangement in a completely randomized design with twelve treatments, three replicates and ten birds per replicate. When the chicks were 42 d of age, 2 chicks (male and female) were selected randomly from each replication (cages) and slaughtered. The liver, heart, gizzard, abdominal fat pad, breast and the femur were immediately weighed. The carcasses without feather, head, feet and internal organs were weighed. All Data were analysed by SAS (2000). Means compared by Duncan's test ($P < 0.05$).

Results The results of this experiment (Table 1) indicated that use of RSM decreases breast and abdominal fat weight of broilers ($P < 0.05$) and had no effect on the Ca and P content of plasma ($P > 0.05$). With the exception of abdominal fat weight, no significant difference was observed with phytase for carcass weight and carcass component weight. There was not any significant interaction between SBM and RSM for effect of enzymes.

Table 1 Carcass weight, carcass components weight (kg) and blood-Calcium and Phosphorous of broilers fed different levels of enzyme and RSM

Variable	Carcass	Breast	Femur	Gizzard	Abdominal fat	Liver	Heart	P mg/dl	Ca mg/dl
No Phytase	1.41	0.44	0.44	0.047	0.032 ^b	0.058	0.009	6.01	8.82
Phytase	1.43	0.45	0.44	0.050	0.037 ^a	0.047	0.008	6.38	8.76
SEM	0.03	0.009	0.01	0.001	0.001	0.001	0.0003	0.15	0.31
No Grindazyme	1.41	0.44	0.44	0.048	0.032 ^b	0.044	0.008	6.22	8.85
Grindazyme	1.44	0.45	0.44	0.049	0.037 ^a	0.46	0.009	6.17	8.72
SEM	0.04	0.009	0.01	0.001	0.001	0.001	0.0003	0.15	0.31
0% RSM	1.47	0.47 ^a	0.46	0.050	0.037 ^a	0.047	0.009	6.15	8.18
25% RSM	1.42	0.45 ^{ab}	0.45	0.048	0.032 ^b	0.047	0.009	6.24	9.13
50% RSM	1.38	0.43 ^b	0.42	0.048	0.035 ^b	0.43	0.009	6.19	9.05
SEM	0.04	0.01	0.01	0.001	0.001	0.001	0.0003	0.19	0.36

SEM: standard error of means; RSM: Rape seed meal; means with different letters differ significantly ($P < 0.05$)

Conclusions Under the conditions of this study, higher levels of RSM showed adverse effects on carcass traits. The results showed that the enzymes were no more effective in the RSM diets than in the control diet without RSM. Further investigations are required to clarify these ideas.

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Food and feed security

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Food security is a global issue. General acceptance of the UN prediction that the world must increase food production by at least 50% in the next 20 years, and at least 100% in the next 40. Climate change and water availability will make this extremely challenging.

There has been a tendency to believe that the UK can rely on trade to ensure its own food security, and that home production is of secondary importance. Trade is an essential element but this analysis is faulty on a number of counts: a) climate change is likely to make Northern Europe a more favoured production area b) the 2007/08 world food crisis showed that the world trading system is not reliable c) relying on increased imports shifts the food security problem on to less developed countries and is of dubious morality.

The UK could increase its agricultural production and, at the same time, continue to improve its environmental performance. But to do this we need several things to happen.

The decline in scientific research and development in agriculture needs to be reversed.

We need to have a new approach to animal health. The NFU has campaigned to take the politics out of animal health, and advocates an independent body, comprised mainly of farmers and professionals. We must eradicate diseases such as TB.

- Relations in the food chain need to improve
- We need to ensure that we have sufficient labour and skills.
- We must avoid inappropriate regulation
- The licensing and approval of GM varieties must be improved, or we will have a crisis in the feed sector.
- There needs to be a culture change in government

Efficient adaptation to climate change: Who adapts, how and when?

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Climate change is characterised by uncertainty. But available economic assessments (e.g. Stern 2006) suggest that delaying action on emissions will lead to increasing global damage cost, with greatest incidence in poorer and vulnerable communities and sectors. There are thus efficiency and equity arguments for early coordinated global action on emissions reduction. In the UK the Climate Change Committee has been established to develop a route map for the “decarbonisation” of growth in all sectors including agriculture.

The cost-benefit picture is less clear on adaptation. High damage burdens in developing countries provide ethical arguments for collective action and aid flows to facilitate the development of adaptive strategies. Elsewhere, the decision to adapt is largely private, and dependent on the interpretation of any downscaled climate data. Climate projections are hedged with uncertainty, which handicaps the development of prescriptive planning. In some sectors (e.g. agriculture) there is a shortage of easily interpretable downscaled information, and conflicting information on whether and when damages may occur. With incomplete information choosing not to adapt is a rational short term option. This is true even when the losses are private.

We know that agriculture is particularly exposed to climate and is an early indicator of adaptation needs. Drawing on a Defra-funded research (AC0307), this paper considers what we know about impacts on the livestock sector, the nature of adaptations, and the role of public and private sectors. The private and public distinction is particularly relevant. There are two perspectives for conducting cost-benefit analysis of adaptation options. Efficient private adaptations may give rise to ancillary public good costs in terms of water pollution, biodiversity and animal welfare. The public sector (i.e. government) therefore has a wider perspective on the costs and benefits of how adaptation happens. At the very least there is a requirement for private adaptations to be synchronised with mitigation measures. The paper concludes by suggesting research avenues for improving the prospects of an efficient adaptation plan for the livestock sector.

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Climate change impacts on the livestock sector - AC0307

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Altered patterns of helminth parasitic disease in Scottish livestock – a role for climate change?

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Introduction The study of the potential impacts of climate change on animal health and welfare is in its infancy and urgently needs to gather momentum. This presentation aims to provide a general overview of the current state of affairs, drawing on available information from a Scottish perspective. There is now a broad consensus amongst scientists that the global climate is changing. All available predictions would indicate that, over the next decade, the weather in the UK will feature greater extremes of climatic conditions, with a general trend towards drier, warmer summers and milder, wetter winters. These changes will obviously impact on the health and welfare of farmed animals, both directly and indirectly. For example, we may anticipate having to protect animals from heat stress and dehydration or provide adequate shelter in more adverse weather conditions. Similarly, there may be indirect effects on livestock through availability of feed supply or a change in forage available for grazing, for example. However, where a changing climate is likely to have most impact is in the transmission and epidemiology of livestock disease. There has been much publicity surrounding the incursion of “exotic” diseases such as Bluetongue and West Nile virus and the spread of vector-borne disease such as Lyme disease and tick-borne encephalitis. However, endemic diseases have been largely ignored. Endemic infectious organisms likely to be strongly influenced by changes in the weather are the parasitic helminths, the causative agents of such diseases as parasitic gastroenteritis (PGE) and liver fluke disease. The larval stages of these parasites develop and survive at pasture and, in some cases, in an intermediate host for a considerable amount of time before being ingested by a host. If climate change alters the ability of some of these larval stages to over-winter on pasture, or survive arrested within the host, the epidemiology of these parasites may be altered accordingly and unexpected, ‘unseasonal’, clinical disease may be witnessed.

Materials and methods This study aims to combine surveillance data with clinical observations and on-farm case studies to investigate the changing pattern of helminth disease in Scottish livestock in the context of climate change and to relate observed patterns to available regional climatic data.

Results A detailed study of the Scottish climate over the past ~30 years reveals that temperature has increased (average, max and min); average precipitation has increased, with some extreme events; the number of frost days has fallen in all regions and the grazing season has been extended by up to 4 weeks in all regions (Barnett *et al*, 2006). These factors all conspire to extend the parasite season and we have seen changes in the prevalence, seasonality and geographical distribution of the key helminth parasite species affecting livestock fitting this picture. For example, there has been a significant increase in the prevalence of liver fluke infection in both sheep and cattle. There is also evidence of a west-to-east spread of disease and the seasonal pattern of infection has become less well defined, such that acute fluke is increasingly diagnosed in early spring and subacute/chronic disease, normally a feature of late autumn/early winter, is increasingly observed in late summer. Similarly with PGE, there has been a significant increase in diagnoses in both sheep and cattle. Heavy infestations of the brown stomach worm *Teladorsagia circumcincta* are now routinely diagnosed in spring when, traditionally, spring teladorsagiosis would have been extremely rare. Furthermore, there have been increasing reports of a northwards spread of outbreaks of disease associated with the highly pathogenic, blood-feeding, nematode, *Haemonchus contortus*. In the only objective study to date addressing potential climate change impacts on parasitic disease diagnosis in the UK, Van Dijk *et al* (2008) performed a rigorous statistical analysis of submissions to VLA and SAC Veterinary Investigation Centres in relation to regional meteorological data. The authors clearly showed that, after identifying and analysing possible sources of bias, the effect of climate change on parasite epidemiology proved to be the most likely explanation for the observed patterns, although other hypotheses and explanations could not be refuted.

Conclusions The climate in Scotland has changed significantly over recent years and we have observed concurrent changes in the prevalence, seasonality and geographical distribution of the key helminth parasites affecting livestock. However, there are a number of confounding factors, other than climate change, which could account for the observed changes. These include the emergence and spread of anthelmintic resistance, the large-scale movement of animals and these parasites’ inherent ability to adapt and change to exploit new opportunities such as those presented by climate change. Frameworks quantifying links between disease trends, climatic change and confounders are urgently needed. In future, the reliance of farmers and practitioners on a blueprint for parasite control in such a changing environment is unsustainable. We need active surveillance and accurate regional parasite forecasting in order to remain vigilant and adapt to the threat posed by helminth parasites.

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Climate change, biochar and carbon sequestration; the potential role for livestock in this scenario?

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Carbon sequestration is seen by many scientists and politicians to be the answer to the problems of both global warming and future supplies of energy. Two main strategies are being considered. One requires the stripping out of carbon dioxide from the waste gases of power stations using coal as feedstock and storing it underground or under the sea bed. The other approach, which is the subject of this paper, is based on experiences in Amazonia thousands of years ago, in which indigenous tribes “created” soil by enriching it with charcoal and human and animal residues (Glaser 2007). “Terra preta” as it was called has been found to have multiple properties. The carbon derived from the charcoal is not oxidized by soil micro-organisms, hence its capacity to “sequester” carbon; of equal importance are properties ranging from being a source of soil nutrients per se and enhancement of the utilization of nutrients that may be added, especially nitrogen (Lehmann *et al.* 2006).

It is the potential to sequester carbon that has attracted most attention by both scientists and politicians with predictions for annual storage rates of biochar in soils equivalent to some 10% of the total global fossil fuel carbon emissions (Lehman *et al.* 2006). Such predictions have also fuelled criticisms from environmentalists and related interest groups that the strategy will result in more deforestation (to provide the biochar) and reduced food production, as land is diverted from production of food crops to specialized “carbon-rich” sources of biomass.

With politicians everywhere desperate to promote carbon mitigation strategies it is not surprising that the predictions for “saving the planet” by carbon sequestration far exceed the basic data on which they are based, nor do they consider exactly how and where the biochar will be produced and how it will impact on the increasing need for food and energy.

This paper describes an ongoing process in which carbon sequestration with biochar is one component of an environmentally friendly integrated tropical farming system, the main objective of which is to produce food and energy from biomass, with live stock playing a pivotal role.

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Equine stereotypes: Current theories and future directions

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Spontaneous stereotypic behaviour is conceptualised as topographically invariant, repetitive, rigid behaviour patterns, typically thought to be an artefact of captivity (Mason and Rushen 2006). In horses, stereotypic behaviours are commonly reported, probably owing to the high numbers of domesticated animals maintained under restrictive conditions (Parker *et al.*, 2008a; Waters *et al.* 2002). Spontaneous stereotypes displayed by the horse include both oral (e.g., cribbing/windsucking) and locomotor (e.g., weaving and box-walking). There are a variety of extant theories as to what causes these behaviours, ranging from boredom to genetic predisposition and everywhere in-between. Affecting up to 35% of domestic horses in the UK, stereotypes represent a significant challenge for owners and a clear understanding of their aetiology is important in order to move to reduce the incidence. Recent advances in our understanding of the behavioural and neurobiological processes involved in the development and maintenance of drug-induced stereotypes in laboratory animals may provide us with a useful model for a better understanding of the spontaneous equivalent.

At present, it appears that an interaction between stress (related to species-inappropriate environmental conditions) and genotype may be the key factors in the development of stereotypes (Cabib and Bonaventura 1997). Recent research has highlighted a neural phenotype in crib biting horses, with altered dopamine (DA) transmission within specific areas in the mid-brain being cited as a mediating aetiological factor (McBride and Hemmings 2005). However, the behavioural and learning affects associated with these reported neurobiological differences are yet to be determined.

We carried out a number of studies, designed specifically to examine some of the behavioural phenotypes associated with crib biting. In the first study, stereotypic horses and controls were trained to choose between two mutually-exclusive schedules of reinforcement, one associated with a short delay to gain food and one with a longer delay. Chronic amphetamine exposure leads to a decrease in sensitivity to delay, and we demonstrated that stereotypic horses showed similar patterns, failing to choose the shorter schedule (Parker *et al.*, 2008b).

In a second set of studies, crib biting and non-crib-biting horses were trained to locate food in a cross-maze. After training, subjects were challenged with a probe trial, in which they were placed in an immediately adjacent mirror image maze. Stereotypic horses were found to shift to stimulus-response learning, an effect typically seen after extensive periods of learning. The groups were then tested in a similar cross-maze; this time, however, the subjects were forced always to turn the same way. In this context, stereotypic horses learnt the task faster (Parker *et al.*, In press). Taken together, the results of the studies indicate that behaviourally speaking, crib-biting horses display similar behavioural phenotypes (insensitivity to delay, and increased habitual responding) to those displayed by animals subjected chronically to psychostimulants. The latter findings also suggested an imbalance of the constituent cells that form the striatum: the striosome and matrix components. If this is found to be the case in future research, this imbalance may hold the key to identifying the aetiology of stereotypic behaviour.

In this talk, I will describe the research in the context of equine learning and welfare.

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Current theories on the pathophysiology of equine laminitis – the link between the gut and foot

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Equine laminitis is a painful debilitating condition that seems to be a product of domestication of horses associated with the practice of feeding carbohydrate rich pasture in excess of requirements, particularly in ponies. We have been working on the hypothesis that caecal bacteria, when presented with carbohydrate in large quantities tend to ferment this. Fermentation by gram positive bacteria is associated with a fall in pH of the caecal liquor. As a means of providing themselves with increased intracellular buffering capacity, these bacteria produce amino acid decarboxylase enzymes which convert amino acids present in the caecal fluid into primary amines which can act as intracellular buffers. The reason we thought this might be the case was that foods formed by bacterial fermentation processes (e.g. cheese and wines) have a high content of amines. Primary amines derived from some amino acids have vasoactive properties. In the 1960s it was recognised that human patients taking drugs that inhibit the metabolism of amines (monoamine oxidase inhibitors) could suffer from severe hypertensive crises if they ate cheese or drank red wines with high tyramine content. This so called 'cheese effect' is the result of the indirect sympathomimetic effect of tyramine which displaces norepinephrine from sympathetic nerve endings leading to a rise in blood pressure. Monoamine oxidase enzymes present in the epithelial cells lining the gut wall and in liver cells normally protect individuals from suffering these hypertensive crises. We wondered whether amines released from the gut in response to ingestion of excessive amounts of carbohydrate might lead to a similar reaction in ponies whereby dietary amines caused vasoconstriction of the digital vasculature, leading to the prodromal phases of laminitis.

Over the last 10 years we have gathered circumstantial evidence that the amine theory may contribute to laminitis. We showed that amines are present in the caecal fluid of horses and ponies, the concentrations being related to diet and increasing when carbohydrate rich pasture is consumed. We also showed that feeding an *in vitro* model of the equine caecum with starch and inulin led to concentration and time related increases in concentration of vasoactive amines and gram positive bacteria proliferated and the pH decreased. The amines produced in the caecum could be measured in the plasma. Furthermore they were found at higher concentrations in the systemic circulation in ponies turned out onto spring/summer pasture as compared to when the same ponies were fed winter pasture supplemented with hay. In addition, dietary amines can stimulate the release of 5-hydroxytryptamine from platelets and cause constriction of isolated digital blood vessels. In some cases, the plasma concentrations of these amines in the spring/summer are close to the threshold for causing constriction of the digital circulation. Infusion of some of these amines into the systemic circulation was associated with a reduced digital blood flow and a concomitant rise in plasma 5-HT concentration and inhibitors of 5-HT receptors have been shown to prevent dietary amine-induced digital vasoconstriction.

These data, whilst interesting, do not prove the link between these phenomena and pasture-induced laminitis. Nor do they explain why some ponies are prone to recurrent bouts of laminitis whereas others eating exactly the same pastures year after year never suffer from this debilitating problem. The underlying phenotype of a pony prone to laminitis has recently been suggested to be associated with a resistance to the hormone insulin. The insulin resistance appears to become much more pronounced as the laminitis prone ponies eat carbohydrate rich grass in the spring-summer months. Laminitis prone ponies seem to have higher blood pressure than their 'normal' counterparts in these months whereas in the winter months there is no difference between these two groups in their blood pressure. Furthermore, laminitis prone ponies have higher circulating triglyceride and uric acid concentrations when compared to their non-laminitis prone counterparts eating the same pasture. These observations are substantiated by a number of different groups working on laminitis throughout the world yet the reason for the association between this metabolic phenotype (which shares some similarities with 'metabolic syndrome' in people) remains to be determined. It is interesting to speculate that the insulin resistant state causes a state of endothelial cell dysfunction, predisposing these ponies to excessive vasoconstriction and platelet activation in the digital circulation when the circulation is exposed to more amines absorbed from the caecum as a result of rapid ingestion of carbohydrate. It is also interesting to note that the endothelium is involved in amine metabolism by an enzyme called semicarbazide sensitive amine oxidase (SSAO) which preferentially metabolises primary monoamines, including one that is not vasoactive but is highly abundant in the caecum, namely methylamine. When the endothelium metabolise primary monoamines, such as methylamine, it produces ammonia, hydrogen peroxide and the corresponding aldehyde of the amine – in the case of methylamine this is formaldehyde. The amine metabolites can be damaging to the endothelium and contribute to endothelial cell dysfunction. Intriguingly, SSAO expression is known to increase in other species in association with the diabetic state where its upregulation has been associated with the vascular complications of diabetes.

To investigate this possibility we have examined endothelial cell function *in vitro* in equine digital veins and arteries following incubation with non-vasoactive primary monoamines, such as benzylamine and methylamine. Preliminary data suggests that some products of the metabolism of these amines can interfere with endothelial-dependent relaxation, particularly when the nitric oxide system has been partially inhibited. Further work is necessary to investigate this phenomenon further and to determine methods whereby endothelial cell function could be assessed *in vivo* to test the hypothesis that ponies predisposed to laminitis have endothelial cell dysfunction that is related to their susceptibility to laminitis. In summary, a range of trigger possible trigger factors continue to be investigated in laminitis research. Dietary amines remain possible candidates, although definitive proof awaits prospective interventional clinical trials.

Milk and meat in our diet: Good or bad for health and wealth?

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Introduction Although globally the demand for animal derived foods is growing rapidly driven by a combination of population growth, urbanisation and rising income, the contribution of milk and red meat to the UK diet has reduced considerably over the last 50 years. The contribution of poultry meat however, has increased enormously. Mainly because of their major contribution to saturated fatty acid (SFA) consumption, ruminant-derived foods have often been considered to be a major contributor to CHD/CVD and other chronic disorders. Given that we are living in an era of increasing risk of chronic disease as a result of increased obesity and an ever ageing population, it is important that risks and benefits associated with different foods are understood and that possible improvements to such foods are made with regard to the cost effectiveness and environmental impact.

Epidemiological evidence A recent meta-analysis (Elwood *et al.*, 2008) of 15 studies showed the relative risk of stroke and/or heart disease in subjects with high milk or dairy consumption was 0.84 (95% CI 0.76, 0.93) and 0.79 (0.75, 0.82) respectively, relative to the risk in those with low consumption. Four studies reported incident diabetes as an outcome, and the relative risk in the subjects with the highest intake of milk or dairy foods was 0.92 (0.86, 0.97). Thus set against the proportion of total deaths attributable to the life-threatening diseases in the UK, vascular disease, diabetes and cancer, the results of meta-analyses provide evidence of an overall survival advantage from the consumption of milk although the situation with other dairy foods is less clear. A positive association between increased milk consumption and prostate cancer was seen however (Givens *et al.*, 2008) and this should not be ignored. Similarly confirmation by WCRF/AICR (2007) of an increased risk of colo-rectal cancer in consumers of red and processed meat needs attention not least to assess the relative risks associated with different so-called red meats.

Saturated fatty acids Despite the recognised benefits of limiting SFA intake in the UK, men and women currently exceed the current mean population target for SFA intake (<11% of total food energy) by 22 and 20% respectively. The proportion of UK children exceeding the target is even greater. Manipulating the fat composition of ruminant-derived foods holds considerable potential as a means of reducing SFA intake and providing health benefits. Use of *cis*-MUFA rich oils in the diet of the cow can reduce SFA in milk fat typically from 70 to 55-60% of total fatty acids, whilst increasing *cis*-MUFA from typically 20 to 33% of total fatty acids. If applied to all UK milk, this would reduce SFA entry into the food chain by some 90,000 tonnes per year and would provide additional benefits to the environment through reduction of methane produced by dairy cows. The health benefits of this SFA replacement strategy is supported by impact modelling (Givens, 2008) and by human intervention studies though more intervention studies are urgently needed with more functional outcomes being assessed.

n-3 fatty acids The beneficial effects of long chain (LC) (carbon chain ≥ 20) n-3 PUFA are well documented but recent evidence indicates that few people achieve the UK daily recommended intake for adults of 450 mg of EPA + DHA per day. In many parts of Europe the daily intake of EPA + DHA by adults and especially young people is less than 100 mg per day, since many never eat oily fish. Poultry meat contributes small but worthwhile amounts of EPA + DHA and studies to enrich the EPA + DHA content of this food will be described and how this would impact on habitual intake. There is also considerable concern that intake of n-6 PUFA has increased excessively over the last 40 years and is aggravating the effects of low LC n-3 intake. Animal-derived foods share some of the responsibility for the increased consumption of n-6 fatty acids and the particular role of poultry meat products will be examined. Substantial changes in the use of oils in the food chain will be needed to deal with this issue.

Cost-benefit. Currently CVD alone is estimated to cost the EU about €170-190 billion per annum and is likely to rise due to an ageing population. A recent cost-benefit analysis in the Lipgene project of reducing SFA intake through dairy cow nutrition indicates that a positive outcome via reduced health care costs would be reached within five years. Thus the economic scope for some dietary change would appear to be large though does need more complex models.

Conclusions Traditionally meat and milk have been traded as commodities with little or no attention to their nutritional or more diverse health value. Given the crucial focus that there must be on dietary improvement such a trading arrangement seems redundant. Ultimately the direction of any population-directed diet modification will depend on cost-benefit analysis and political will. A major political challenge will be the redirection of health care savings back to support changes to primary food production.

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Feed additives – over-regulation or measured response?

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A number of major food safety incidents in the 1990's led to a crisis of confidence in EU consumers. These included the Mad Cow Disease outbreak, starting in the UK in the 1980's, and the scandals across Europe involving the feeding of dioxins, sludge and pharmaceutical waste to food-producing animals. These incidents had one thing in common – all were connected with animal feed, suggesting this was the weak link in the food chain. In addition, the food industry continued to struggle with the negative publicity of salmonella, *E. coli* and campylobacter incidents.

In response to this consumer confidence crisis, or more likely to the ensuing political outcry, the European Commission introduced a White paper on food safety. This White paper looked at a three-pronged approach to food safety, suggesting a full range of food safety laws, backed up with a new independent European Food Safety Authority (EFSA) and effective controls and enforcement. This was followed by a raft of legislation, starting in the early 2000's and continuing to this day.

The initial piece of relevant legislation was the Food Law (Reg. 178/2002), which established EFSA as an independent, scientifically-based organisation. This Regulation introduced the principles of risk analysis, traceability, labelling, the precautionary principle and business operator responsibility. It also defined the separate functions of risk management and risk assessment, the former carried out by the political institutions of the EU such as the Member States and the Commission, and the latter by an independent and transparent EFSA.

The next piece of key legislation was Regulation 1831/2003, on Additives for use in Animal Nutrition, which covered feed additives and premixtures sold in the EU. This essentially brought all feed additives for the first time under one piece of legislation, and excluded antibiotics (other than coccidiostats and histomonostats). The Regulation defined the purpose of additives and outlined five categories; technological additives, sensory additives, nutritional additives, zootechnical additives and finally coccidiostats and histomonostats. It also defined holder-specific authorizations for those first placing on the EU market zootechnical additives, coccidiostats or histomonostats or any additives derived from genetically modified organisms. This placed extra demands on the applicant to provide additional information relative to the non-holder specific authorizations, but also protects the applicant by preventing others from selling under this approval. Non-holder specific authorizations would essentially allow sales of a similar product by competitors.

The first effect of Reg. 1831/2003 was to oblige all additive suppliers to notify the EU Authorities within 1 year (i.e. before November 2004) of all additives which were on the market. Based on this, the Commission then published a register of all allowed feed additives in November 2005, and has kept this document updated with any subsequent changes. Suppliers were also obliged to provide full registration dossiers for all these additives before November 2010. Seven years from first publication seems quite a long time for industry to provide full dossiers; however, the guidelines on how to build and assess a dossier were not published until April 2008 (Reg. 429/2008), leaving time rather short to plan, execute and report trials to prove quality, safety and efficacy of what for a number of companies is a portfolio of divergent products.

Based on the present register of over 2000 products and in the knowledge that each product will have several suppliers, it becomes clear that the Authorities could be presented with several thousand dossiers for review in November 2010. This puts an enormous burden on both the companies and the Authorities. In an attempt to streamline this process, the suppliers, through FEFANA (the European Association of Feed Additive and Premixture Producers), have formed consortia of companies active in specific areas to help streamline this process. These consortia will attempt to ensure that non-holder specific dossiers are of adequate quality and that industry will, where possible, only submit one dossier per product. The Authorities have also agreed that, where appropriate, similar products can be represented by one dossier. FEFANA are also discussing how to deal with "orphan" additives which are necessary for animal health, productivity or welfare but have no commercial sponsor willing to develop a dossier. There is still a lot of work to be done before and after the deadline of November 2010!

The next piece of legislation to strongly influence feed additives was the Feed Hygiene Regulation (1831/2005). This clearly stated that the primary responsibility for safety rests with the industry. In many cases industry had already taken responsibility, with quality assurance schemes such as FEMAS and UFAS already in place in the UK. FEFANA had also developed a parallel QA scheme (FAMI-QS) for additives and premixtures, and this was formally approved by the EU Standing Committee on Food Chain and Animal Health in January 2007. FAMI-QS is now widely used both within the EU and outside by additive and premixture operators to ensure product quality and compliance with the Feed Hygiene Regulation.

It is inevitable that we will see additional EU legislation in the feed area over the next few years, both updating present regulations as well as introducing new challenges. We are also likely to see feed industry continue to take greater responsibility for product and food chain safety and quality. However, it is unlikely that the radical changes we have experienced over the past decade will be repeated.

Animal feed, and in particular feed additives, within the EU is today the most regulated area of the food chain in the world. However, these regulations are widely accepted and supported by the European feed additives industry – we are all consumers and have a vested interest in assuring the safety of our food! Further, as a direct result of these regulations the EU feed additive industry is in a much better position to satisfy the safety and quality concerns of customers and consumers worldwide. However, despite all the recently introduced legislation, we will continue to see both avoidable and unavoidable food safety challenges.

Zoos: Conservation or exploitation?

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Introduction Captivity should be the last resort in any attempt to conserve threatened species. However, for many species we are now at this point, particularly when we consider the lower vertebrates such as amphibians. Zoos have the potential to play a leading role in the conservation (as opposed the exploitation) of this often-neglected taxa. However, in order to do this will require two significant mind-shifts within the zoo world. Firstly we need to distinguish between those species which we maintain for exhibition purposes (i.e. large mammals) from those which have an important conservation role (e.g. amphibians) and be willing to discuss this openly with our visiting public. Second we must recognise that while educating children about the natural world is of value, our biggest impact on wildlife conservation will be through training conservation professionals worldwide and building their capacity to conserve their own wildlife.

Summary In an ideal world we would have no need for zoos. Gerald Durrell, founder of the Jersey Zoo and the Durrell Wildlife Conservation Trust, once said that he looked forward to the day when we could close down his zoo as there would be no need for it. The reality is that few zoos are making significant contributions towards tackling the current extinction crisis. Many captive animals are now within organised, international breeding programmes which arguably reduce zoo's reliance on restocking from the wild. However, whether or not the establishment of studbooks should fall under the umbrella of species conservation is open to question. Due to behavioural change, genetic impoverishment and loss of wild 'instincts' these captive populations are of limited value in any proposed reintroduction programme.

Zoos do provide an unrivalled opportunity to educate the general public about the state of the natural world and how we need to modify our own behaviours to reduce further biodiversity loss. Children are a common focus for zoo conservation education departments. While it is clearly important to influence the next generation of decision makers we need behavioural change now if we are to challenge the current rate of species loss on the planet. What then should zoos do if they are to make a significant contribution to the conservation of life on Earth?

The Durrell Wildlife Conservation Trust, based in Jersey (British Channel Islands) was established by Gerald Durrell with the mission to "*save species from extinction*". From the outset the organisation sought to identify those species which were outside the conservation spotlight. We focused on what Gerald Durrell referred to as "*Little Brown Jobs- LBJ's*". Amphibians are one important group of LBJ's. With one in three amphibians now considered at risk of extinction they are in greater need of conservation intervention than ever before. Amphibians lend themselves to the captive environment in being relatively small, quick to breed and most importantly comparatively unaffected by the captive environment from a behavioural point of view. Through our involvement in conservation projects for the Mallorcan midwife toad, Jersey agile frog and Montserrat mountain chicken we have been able to demonstrate that zoos can play a critical role in species conservation and applied research in support of wild populations. With the establishment of bio-secure units onsite we are now well-placed to begin breeding and research programmes for a variety of threatened amphibians.

Another important development which Gerald Durrell believed in passionately was the International Training Centre (ITC), lying adjacent to the zoo. Since its establishment in 1984 the ITC has trained over 1700 conservation professionals from more than 120 countries in endangered species recovery techniques. In 2006 the ITC in collaboration with the Herpetology Department at Durrell launched its first Amphibian Biodiversity Conservation (ABC) training course. This course has now been run in Jersey, India and Bolivia and has trained over 70 amphibian conservation biologists. In 2008 we developed a second course focusing on Amphibian Conservation Husbandry (ACH) to help build capacity within the world's zoos for the captive breeding of threatened amphibians. In August we hope to take this course to Sri Lanka. This training is beginning to equip conservationists worldwide in the skills they need to conserve amphibian life.

In summary, zoos do have the potential to play a significant role in biodiversity conservation. However, I would argue that in order to realise their full potential in this field zoos need to give greater attention to the lower vertebrates in our collections (i.e. away from the mega-vertebrates). Furthermore we should divert more of the resources we currently apply to teaching children towards training more conservation biologists from the countries of the world in greatest need of conservation action.

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Production and other ecosystem services from extensive grassland-based systems in Europe: policy options and technical innovations

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Extensive systems Extensive agricultural systems are usually presented as a type of agriculture that uses low quantity of inputs per surface unit (land), like fertilizers, feeds, seeds, pesticides, irrigation water and energy. They are frequently associated with low investments (capital) in machinery and/or buildings per surface unit and low land prices. They can also be characterized by labour-extensive systems. European extensive grassland-based systems are mainly located at high latitudes (Scandinavia) and high altitudes (mountain areas), in Mediterranean dry rangelands, in the lowlands of the Eastern part of the Union (recent accession countries), as well as in hills and uplands of the British Isles. Grasslands and rangelands covered 56 million ha (33% AA) in the EU-27 in 2005. This area included about 17.5 million ha of rangelands, in mountain and Mediterranean areas mainly (EUROSTAT, 2008). Extensive grasslands and rangelands cover thus more than 30% of the total grassland and rangeland area in Europe. Forage yields and animal productions per ha are significantly lower in these areas compared with intensive lowland grasslands. For instance an extensive range can produce about 5-6 times less DM/ha than fertilised grasslands. Production costs per kg of product are often significantly higher in extensive systems. Farmers must manage larger areas in these systems for a total income that is regularly lower compared with smaller intensive farms.

Ecosystem services The Millennium Ecosystem Assessment (MEA, 2005) identified 4 types of services: (i) provisioning services such as food, feed, fibre and fuel and also secondarily, water and timber supplies; (ii) regulating services that affect climate, floods, disease, wastes and water quality; (iii) cultural services that provide recreational, aesthetic and spiritual benefits; (iv) supporting services such as soil formation, photosynthesis, nitrogen fixation and nutrient cycling. These services are provided by the combined effects of the 5 environmental resources (air, soil, water, energy and biodiversity) but mainly by biodiversity. Intensive European systems are able to ensure a regular provision of food and feed in high amount and of good quality, but they have negative effects on almost all other ecosystem services. Extensive systems have the opposite effects. They were able to create diverse, high-quality landscapes and to create opportunities for biodiversity to develop (High Nature Value farmlands). They also produce usually high-quality products that are more healthy and tasty than products from intensive systems.

Policy options The main agro-ecosystem services and environmental issues in agriculture are food security and safety, soil protection, water quality and availability, climate change, animal welfare, biodiversity conservation, landscape quality and recreation opportunities. Public demands on agricultural land and especially on grasslands are related with these multiple functions and values. Extensive systems are particularly efficient for soil protection, water quality and availability, biodiversity conservation, landscape quality and recreation opportunities. Though, the financial value of the ecosystem services has still to be evaluated and taken into account. It is all the more difficult to do that most environmental resources are public goods. Most services provided by agro-ecosystems and thus partly by the action of farmers are not rewarded yet. Farmers are thus not encouraged to take care and to maintain these services. As a consequence, they are progressively degraded. Political decisions and programmes are needed for correcting this natural trend of the market of private goods on the degradation of the value of public goods. The European Common Agricultural Policy (CAP) supports extensive systems through its 2nd pillar (rural development policies). This includes several programmes such as Less favoured Areas (LFA) payments and Agri-environmental measures (AEM) including the support to Organic Farming. The new Rural Development policy 2007-2013 includes an axis called 'Leader' which introduces possibilities of direct support to locally based bottom-up approaches (EUROPA, 2007). The EU developed a Product Quality Policy that includes the 'Protected Designation of Origin' (PDO) system. Through this system, farmers can increase their income by selling high-quality cheeses and meat at a higher price compared with conventional products. The CAP reform of 2003 introduced in the cross-compliance principle the obligation to maintain the proportion of permanent grassland in the AA in order to maintain the land in 'Good Agricultural and Environmental Conditions' (GAEC). The Natura 2000 programme is protecting the grassland area in extensive, species-rich systems. These programmes were though not sufficient to avoid the decrease of the grassland area, its intensification or its abandonment. More efforts should thus be devoted for maintaining the multiple ecosystem services provided by extensive grasslands. This must be done through several means including a financial recognition of the production of public goods and technical supports for farmers.

Innovations In the future, efficient and profitable systems will have to be less energy demanding while conserving biodiversity. Researches should focus on these two topics, for example on energy costs and GHG emissions per production system and per product for developing such energy efficient systems. Nitrogen fixation by legumes will be one of the pillars of these future systems. A special effort will have to be done by livestock systems because they are less energy efficient than arable systems per kg of food produced. There are also some new opportunities for producing agro-fuel in extensive regions.

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Efficient ruminant production from grassland

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Ruminant production in the UK is largely dependent on grasslands: approximately 52% of UK land is (improved) grassland and rough grazing. Herbage production from improved grassland can be over five times higher than that of indigenous swards (Davies *et al.*, 1984) and these pastures account for the majority of ruminant (meat and milk) production from grassland the UK. They are also amenable to manipulation through the choice of species, variety and mixture of forages sown. Efficiency of production can be influenced at the levels of both the plant and the animal and by the nutrition and genetics of both. This paper examines strategies to increase production efficiency based upon optimising rumen fermentation linked to plant breeding approaches. In this way efficiency can be improved without recourse to diet manipulation with supplements, which is generally impractical at grazing. Although the rumen microbial population allows the ruminant animal to extract energy from otherwise unusable sources (i.e. fibres), the rumen is also the source of greatest inefficiencies in the use of nitrogen and energy. Degraded plant nitrogen that is not captured by the rumen microbes tends to be absorbed as ammonia, much of which is excreted as urea and contributes to ammonia and nitrous oxide emissions. Similarly, methane release from the rumen represents a waste of energy that could otherwise be used for production. Globally, these two processes result in ruminant livestock accounting for approximately a third of the methane emissions (Beauchemin *et al.*, 2008) and half of the nitrous oxide emissions (de Klein and Eckard, 2008) from anthropogenic sources.

Improving dry matter digestibility has been, and remains, a key plant breeding objective to increase the nutritive value (and hence efficiency of use) of forage crops. More recently, energy and nitrogen use efficiency have been addressed in breeding programmes in more focussed ways, largely aimed at balancing energy and protein availability in the rumen. Ryegrass breeding aimed at increasing the concentration of water-soluble carbohydrates has generated varieties that can reduce the proportion of dietary nitrogen intake that is excreted as urinary nitrogen (Moorby *et al.*, 2006). Although increasing the ratio of water soluble carbohydrate to crude protein in grass leads to significant reductions in excretion of nitrogen in urine (Edwards *et al.*, 2007), the high concentrations of crude protein required for optimum grass growth are likely to mean that impractically high concentrations of water soluble carbohydrates would be required. Therefore, reducing protein degradability has practical potential to improve rumen protein use efficiency. A number of natural protein protection mechanisms could be exploited to reduce rumen protein availability. Several varieties of important feed crops, including ryegrass, exhibit stay-green mutations whereby senescent material retains chlorophyll and associated proteins rather than mobilising the nutrients for use elsewhere in the plant. Although there was no difference in availability of nitrogen in fresh stay-green ryegrass compared with a standard variety (Kingston-Smith *et al.*, 2002), it has a higher protein content when comparing senescent material (i.e. hay). Various plant enzyme systems and secondary metabolites can improve nitrogen and energy use in the rumen. The enzyme polyphenol oxidase helps reduce proteolysis in ensiled red clover (and may also act in the rumen in freshly ingested material), thus increasing nitrogen use efficiency of the feed in livestock. Its activity in some grasses species (Lee *et al.*, 2006), indicate the potential for its incorporation into other important forages. Condensed tannins present in some forages have been shown to improve the efficiency of capture of microbial protein. In addition, some tannin-containing forage crops (e.g. birdsfoot trefoil) have been shown to reduce methane emissions when grazed (Ramírez-Restrepo and Barry, 2005), although the agronomic characteristics of many would need improving to make their use practical in the long term.

In conclusion, although a host of options are available to increase efficient production of ruminants from grassland, for animals at pasture the most effective options involve the appropriate choice of grazing material. Forages that have been bred for improved nutrient composition and improve energy and protein efficiency in the animal contribute to reducing livestock's environmental footprint while maintaining local food supplies.

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Balancing the competing needs of climate change, biodiversity and rural communities

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The work of the UK Climate Change Commission (UKCCC) in recommending targets and options for reducing emissions of greenhouse gases is focusing attention on what agriculture and land use can contribute to deliver these targets. Although overall the major issue is the reduction of carbon dioxide emissions from energy use, agriculture and land use are significant emitters of methane and nitrous oxide. UKCCC has identified three main routes by which emissions can be reduced

- Lifestyle change with less reliance on carbon intensive produce - eg switching from sheep, and beef to pig, poultry and vegetables.
- Changing farm practices – eg to improve use of fertilisers and manures
- Using new technology on farms – eg modifying rumen processes, anaerobic digestion.

Much work remains to be done to quantify the reductions that can be obtained from these routes, but it is important to recognise that climate change policy cannot be divorced from the wider issues of food security and the growth of interest in sustainable agriculture and agri-environmental programmes. In the last 10-20 years there has been unprecedented critical scrutiny surrounding the nature and development of contemporary systems of food provision, reflecting in part increasing societal concerns over the environmental and food safety/health dimensions of farming practices.

To date, much debate about sustainable agriculture has centred on the farm. In contrast, the two other important developments - shorter food supply chains and consumer influence - are more recent, and relate to the renewed focus on local food and drink products, typically constructed around notions of quality, territory and social embedding. In this context, sustainability is seen in terms of the wider agro-food system, especially the vertical linkages in the food supply chain beyond, but including, the farm. Emphasis on sustainable food chains is an important departure because it involves looking at the life cycle analysis from production to consumption, not just within the farm.

One way to approach the analysis of what agriculture delivers is through the quantifying of ecosystem services. These services are based on the processes by which the natural environment produces resources that are useful to people, including goods like timber and fish, and services such as purifying water, pollinating crops and providing a natural defence against flooding. The Millennium Ecosystem Assessment (2005) provided a framework to value ecosystem services at regional and national levels. It classified ecosystems into 4 categories

- Provisions services i.e. products obtained from ecosystems
- Regulating services i.e. benefits obtained from regulation of ecosystem processes
- Cultural services i.e. non material benefits that people obtain through spiritual enrichment, cognitive development, recreation etc
- Supporting services, necessary for the production of all other ecosystem services.

The cross cutting benefits for eco-system services of reducing greenhouse gas emissions will be presented for Welsh agriculture and land use

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