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**001**

# Immunohistochemical localisation of aquaporin water channels in the bovine mammary gland

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**Introduction** The aquaporins (AQPs) are a family of small integral membrane proteins 28-35kDa in size, sub classified into 2 groups; those selectively permeated by water (the aquaporins; AQP0, AQP1, AQP2, AQP4, AQP5, AQP6 and AQP8) or by water and small organic osmolytes such as glycerol and urea (the aquaglyceroporins; AQP3, AQP7, AQP9 and AQP10). AQPs are expressed in a variety of epithelial tissues where they are responsible for rapid water movement driven by osmotic gradients. The mammary gland produces and secretes milk which consists of water, lipids, electrolytes, vitamins, sugars and specific milk proteins. However, little is known about AQP expression, distribution and function in mammary tissue or about the process of water transport across the mammary epithelium. The only information available relates to the identification of AQP1 and AQP3 in capillary endothelia and epithelial cells of mouse mammary glands respectively (Matsuzaki *et al*., 2005). Since water delivery to the mammary gland is critical for milk production, which is of major economic importance to the dairy industry, it is necessary to understand the underlying physiology involved. The aim of this study was to determine the expression and distribution of AQPs within the bovine mammary gland.

**Materials and methods** Rabbit polyclonal antibodies against rat AQP1, AQP2, AQP3, AQP4, AQP5, AQP6, AQP7 and AQP9 were developed in collaboration with Sigma-Genosys. In most cases, a highly conserved (isoform specific) C-terminal synthetic peptide was used as immunogen. Fresh bovine mammary tissue was collected from local abattoirs. Samples included tissue from the teat, cistern and secretory portions of mammary glands from lactating cows and non-lactating Heifers. These were removed after slaughter and promptly fixed in 10% formalin. Sections (7µm) were cut from these tissue blocks and mounted on charged microscope glass slides. Bovine kidney was used as positive control tissue in all experiments. Immunohistochemistry was then carried out on the sections using a commercially available kit and recently published method (Mobasheri and Marples, 2004).

**Results** Expression of selected AQPs in bovine mammary tissue is summarised in Table 1. In general, AQPs that were expressed in the non-lactating heifer were expressed with increased intensity in the milking cow. This difference in AQP expression was particularly marked in the case of AQP1 where there was increased expression in the tubuloalveolar secretory portion of the gland and in the myoepithelial cells in the underlying connective tissues. A similar observation related to AQP3 and AQP4 although here the difference was most marked in the teat duct epithelia.

**Table 1** Expression of selected AQPs in bovine mammary tissue.

| AQP | Heifer | Cow |
|---|---|---|
| AQP1 | Capillary endothelia in teat, cistern, glandular and adipose tissue sections. Myoepithelial cells underlying teat duct epithelia. | Increased expression in capillary endothelia in teat, cistern, glandular and adipose tissue sections Myoepithelial cells underlying teat duct epithelia. |
| AQP2 | Not expressed | Not expressed |
| AQP3 | Selected epithelial cells in teat, cistern and glandular tissue. Teat smooth muscle bundles. | Increased expression in selected epithelial cells in teat, cistern and glandular tissue. Teat smooth muscle bundles |
| AQP4 | Selected epithelial cells in teat, cistern and glandular tissue. Teat smooth muscle bundles. | Increased expression in selected epithelial cells in teat, cistern and glandular tissue. Teat smooth muscle bundles |
| AQP5 | Glandular tissue. | Increased expression in glandular tissue |
| AQP6 | Not expressed | Not expressed |
| AQP7 | Epithelial cells in the teat and glands. Teat smooth muscle bundles | Increased expression in epithelial cells in the teat and glands. Increased expression in teat smooth muscle bundles |
| AQP9 | Leukocytes within the gland and blood vessels. | Leukocytes within the gland and blood vessels. |

**Conclusions** This study has provided, for the first time, a comprehensive catalogue of AQPs expressed within the bovine mammary gland. The AQPs found in mammary tissues were found in expected anatomical locations. There were marked differences between the non-lactating heifer and the lactating cow in terms of AQP expression and abundance. These results suggest that AQP expression is increased in lactating mammary glands and AQPs may be participants in the mechanism for controlling water content of milk by diluting the protein and lipid contents of milk to an isotonic solution as it descends through the teat duct system. Further work is required to develop a larger bank of mammary tissue samples for more comprehensive immunohistochemical analysis using custom designed tissue micro-arrays. It will also be useful to obtain further information about the animals including genetic merit, milk yield, lactation number, previous cases of udder disease e.g. mastitis and whether the animal was pregnant.

**Acknowledgements** The authors are grateful to BSAS/Genesis Faraday for a vacation scholarship in addition to Dr Kieron Salmon for useful clinical discussions, Lee Moore and Nigel White for their help in procuring abattoir samples.

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**002**

# Detecting dominance QTL in poultry pedigrees using variance component methodology

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**Introduction** Methods that detect QTL within commercial populations circumvent the need for expensive experimental populations and facilitate direct application of results through marker assisted selection. Variance component analysis (VCA) uses phenotypic, pedigree and marker information within a mixed linear model to simultaneously detect QTL and estimate breeding values. The inclusion of non-additive effects has potential for greater accuracy of selection and understanding of underlying mechanisms. The linear model can be extended to include higher order effects such as dominance, however, there is little information on empirical power. Here VCA was applied to real and simulated commercial broiler data to detect additive and dominant QTL effects.

**Materials and methods** Data on 40 day conformation score and bodyweight were available for a commercial broiler dam line. A two-generation pedigree from Cobb-Vantress Breeding Company Ltd of 100 dam families nested within 46 sire families gave a total of 2708 records with marker genotypes available for candidate regions on chicken chromosomes 1,4 and 5. For simulations, a typical poultry pedigree with 20 sires each mated to 5 dams with 20 offspring per dam was simulated together with a 20cM chromosome with 5 markers 5cM apart. A range of additive and dominant QTL effects were simulated to explore empirical power to detect dominance and false positive rates. QTL mapping at 5cM intervals using the VCA method involved estimating IBD coefficients (Pong-Wong *et al.*, 2001) to construct variance/covariance matrices as described by Liu *et al.*(2002). Fixed effects of sex, age of dam and hatch within flock were fitted for the real data. Variance components were estimated using ASREML for nested mixed models including random maternal, polygenic, additive QTL and dominance QTL effects. A test statistic for a given location was obtained using a likelihood ratio test.

**Results** Table 1 shows variance due to polygenic and QTL effects at the highest test statistic. Significant dominance QTL effects were found for weight on chromosome 4 and for conformation on chromosomes 4 and 5. For conformation on chromosome 4 the additive variance is reduced to virtually nil with dominance effects explaining 8% of total variance. Similar effects are seen for weight and conformation on chromosome 5 with dominance effects explaining 6% of total variance.

**Table 1** Proportion of total variance explained by polygenic and QTL effects after fitting polygenic, additive QTL and dominance QTL effects for weight and conformation score

| Chr | Trait | Marker interval | Polygenic effect | Additive QTL | Dominance QTL |
|---|---|---|---|---|---|
| 1 | Weight | *MCW0297-MCW0112* | 0.08 | 0.06 | 0.04 |
| | Conf | *MCW0297-MCW011* | 0.18 | 0.05 | 0.00 |
| 4 | Weight | *ADL0266-LEI0076** | 0.12 | 0.03 | 0.03 |
| | Conf | *ROS0015-ADL0194*** | 0.20 | 0.00 | 0.08 |
| 5 | Weight | *MCW0090-ROS0013* | 0.12 | 0.00 | 0.06 |
| | Conf | *MCW0090-ROS0013** | 0.19 | 0.00 | 0.06 |

Variances at highest test statistic fitting additive and dominance qtl effects versus no QTL effect. * for $P<0.05$ and ** for $P<0.01$.

Main findings from the simulations were: a) dominance variance greater than 4% of phenotypic variance could be detected with power > 90%; b) empirical false positive rates were lower than expected indicating tests are conservative; c) little spurious dominance was found; d) routinely including a dominance component in the model resulted in little loss of power; and e) there is evidence of confounding between maternal common environment and dominance even at individual markers if a maternal component is not fitted in the model.

**Conclusion** To our knowledge this is the first report of dominance QTL detected in commercial livestock populations using VCA. Significant dominance QTL effects were found for weight on chromosome 5 and conformation on chromosomes 4 and 5. At the highest test statistic virtually all of the QTL variance could be explained by a dominance effect. This is an interesting result indicating that VCA was able to detect relatively modest dominance QTL effects under a test shown to be conservative under simulated conditions. Simulations will be extended to explore varying population size and structure.

**Acknowledgements** We gratefully acknowledge Genesis Faraday and the BBSRC for funding of S.Rowe, DEFRA (LINK project LK0625) and Cobb Ltd. for financial support and data collection.

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Liu, Y.,Jansen, G.B.,and Lin, C.Y. 2002. The covariance between relatives conditional on genetic markers. *Genetics Selection Evolution* **34:** 657-678.

**003**

# Novel quantitative trait loci for chemical body composition traits in pigs

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**Introduction** Quantitative trait loci (QTL) associated with physical and chemical body composition of the pig are of substantial economic interest. Previous studies have reported QTL for physical body composition such as lean and fat tissue traits (Roehe et al., 2003). In contrast, QTL associated with chemical body composition and for the change in deposition of such components during growth have only been reported in one previous study (Mohrmann et al., 2006). Knowledge of the genomic regulation of body composition during growth is important to accurately estimate nutritional requirements, optimise the entire production system, characterise the population of interest, and to optimise food intake capacity by breeding.

**Materials and methods** QTL mapping was based on data from a 3 generation full-sib design (Pietrain x commercial dam line). Phenotypic data was available for dissected carcass cuts, carcass measurements obtained from the AutoFOM system and meat quality measured at slaughter weight (140 kg body weight). Data was also available for chemical body composition of the empty body and protein and lipid deposition of live animals throughout growth from 30-140 kg. Additional data was available for daily gain, feed intake and food conversion ratio (FCR) throughout growth. Animals were genotyped for 48 informative molecular markers covering chromosomes 2, 4, 8, 9, 10 and 14. QTL mapping was carried out using least squares regression interval mapping with a web based package called QTL Express (Seaton et al., 2002).

**Results** Novel QTLs have been found for lipid accretion from 120-140 kg associated with numerous QTLs for subcutaneous fat on chromosome 9 and for lipid accretion from 60-90 kg closely located to one QTL for intramuscular fat on chromosome 8 (Figures 1 and 2). QTL for protein accretion 120-140 kg was identified in the same region of chromosome 9 as the QTL for lipid accretion around numerous QTLs for lean and fat tissue traits. Additional QTL were detected for FCR 90-120 kg on chromosome 2 around QTL for body composition traits (Figure 3).

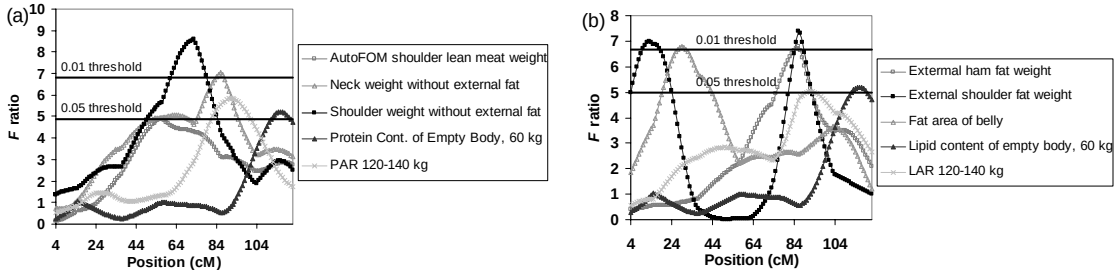


**Figure 1** QTL for (a) protein accretion (PAR) 120-140 kg and (b) lipid accretion (LAR) 120-140 kg detected around QTL for lean and fat tissue on chromosome 9

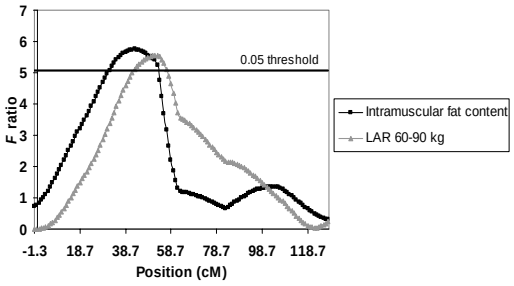


**Figure 2** QTL for lipid accretion (LAR) 60-90 kg in a region of only one QTL for fat tissue on chromosome 8

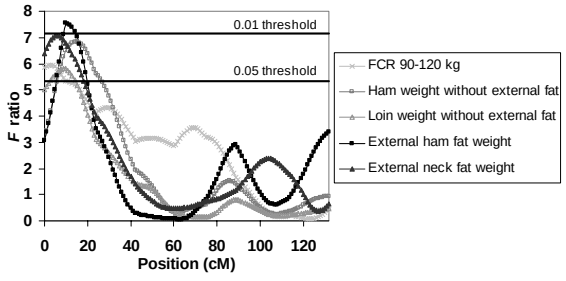


**Figure 3** QTL for FCR 90-120 kg detected around QTL for lean and fat tissue on chromosome 2

**Conclusions** Numerous QTL were detected in this study for physical and chemical body composition. The QTL identified in this study for lipid accretion are unique in the literature. In conjunction with a previous study (Mohrmann et al., 2006), QTL for protein accretion have been identified for all growth stages (30-60 kg, SSC1; 60-90 kg, SSC13; 90-120 kg, SSC1; 120-140 kg, SSC9 in Figure 1). In conclusion, protein accretion is likely regulated differently throughout growth. Body composition is likely to be the cause of the QTL for FCR on chromosome 2, whereas the QTL identified for FCR 60-90 kg in a previous study was caused by increased protein accretion (Mohrmann et al., 2006). In particular the QTL associated with FCR are of high economic interest to be exploited within marker assisted selection schemes.

**Acknowledgments** BBSRC, Genus and Genesis Faraday for their financial support.

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**004**

# Lleyn breed is likely source of BMP15 and GDF9 mutations, that have large effects on ovulation rate, discovered in Cambridge and Belclare breeds

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**Introduction** Exceptionally high prolificacy in the Cambridge and Belclare breeds has been shown to be attributable to point mutations in genes (BMP15 and GDF9) that code for oocyte derived growth factors which have profound effects on ovarian follicle development. The BMP15 gene is X linked while GDF9 is autosomal (chromosome 5). Two different BMP15 mutations are present in the Belclare population (*FecX*$^{G}$ and *FecX*$^{B}$) but only one of these (*FecX*$^{G}$) was found in the Cambridge while the GDF9 mutation (*FecG*$^{H}$) was common to both breeds. The Inverdale and Hanna mutations in NZ Romney sheep (Davis et al. 2001) are point mutations in BMP15 but involve different codons from those involved in Cambridge and Belclare. It is of interest to know whether the mutations common to Cambridge and Belclare are likely to have had a common ancestral source or arose independently. The Lleyn breed was a common source of genetic material in the genesis of both breeds. Thus, the foundation ewes for the Cambridge included three Lleyn ewes (Owen,1976) while a set of 13 Lleyn sheep selected for high litter size was a major contributor to the Belclare breed (accounted for 50% of the genetic material at one stage; subsequently diluted to about 25%). The hypothesis is that the Lleyn was the likely source of the two mutations common to Belclare and Cambridge.

**Materials and methods** The Lleyn sheep that contributed to the development of the Belclare were sourced in 1976 in the Lleyn peninsula of North Wales, the original homeland of the breed. Owners of Lleyn sheep in that area were traced during 2002 through contacts involved in the original importation and were asked to provide blood tissue from any ewes in their flocks that were known to have high litter size performance (≥ quadruplets once or triplets at least twice). Blood was obtained from 43 ewes representing 6 flocks. DNA was extracted using standard procedures and animals that had litter size records were genotyped for both BMP15 mutations and for GDF9 as described in Hanrahan et al. (2004).

**Results** Litter size data were provided for 32 of the ewes and these data are summarised in Figure 1. A litter size of 3 or more occurred in 75% of the records available while 7 ewes had 4 or more lambs at least once. Details available varied; at least three litter size records were available for 13 ewes, 2 records/ewe in 2 cases, while a single record was available for 12 ewes. In the remaining cases the records only indicated that the ewe had produced triplets more than once. In the latter cases a single litter size of 3 was assumed for the summary in Figure 1. The weighted mean litter size was 2.97. DNA analysis showed that 9 ewes were heterozygous carriers of the *FecX*$^{G}$ mutation. No carriers of the *FecX*$^{B}$ were present in the sample while one ewe was heterozygous for the GDF9 mutation (*FecG*$^{H}$). The litter size record in the latter case was 5,6,6. The carriers *FecX*$^{G}$ had an average litter size of 3.0 (range 2 to 5).

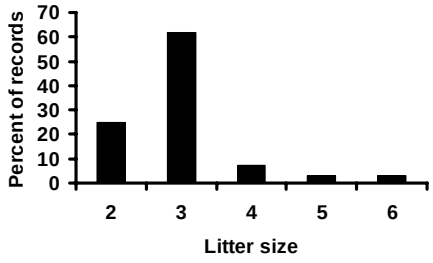

Figure 1. Litter size records for Lleyn ewes

The litter size records of the 13 Lleyn ewes imported into Ireland in the mid 1970s and used in Belclare breed development averaged 3.3 (a litter of 5 was represented in the history of 2 ewes) (Hanrahan, 1976). One of the Lleyn rams imported was born as a sextuplet. The Lleyn ewes contributing to the Cambridge included one with quadruplets (3 times) and quintuplets (once) in her lifetime record (Owen, 1976).

**Conclusions** The presence of both BMP15 (*FecX*$^{G}$ ) and GDF9 (*FecG*$^{H}$**)** mutations in the Lleyn population strongly supports the hypothesis that the Lleyn is the source of these two mutations in both Cambridge and Belclare. The BMP15 mutation (*FecX*$^{B}$) present in Belclare but not in Cambridge, is likely to have been represented among the highly prolific ewes selected from Irish flocks in the 1960s and used to establish the High Fertility line which was part of the foundation stock used to generate the Belclare breed (Hanrahan, 1989). A more extensive survey of the Lleyn population with respect to genotype at the BMP15 and GDF9 loci is desirable given the increased importance of the breed in Britain in recent years and the fact that homozygous carriers of these mutations are sterile (ovarian hypoplasia) due to disruption of primordial follicle development.

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**005**

# Identification of QTL controlling distress at social separation in cattle

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**Introduction** Modern cattle management systems involve animals' separation from penmates for procedures such as weighing, measuring and blood-sampling. This separation can result in distress in some individuals. Environmental and genetic factors are known to control the variability observed in behavioural responses of different individuals. We carried out a genome scan in a Holstein x Charolais cross population to identify QTL influencing temperament-related traits. One of these tests was the social separation test (SS test), which was designed to measure animals' fearfulness to social separation (Ball *et al.,* 2002). We present here the results obtained for the traits scored in the SS test performed in the heifers of the second-generation of this population.

**Materials and methods** *Resource population:* The RoBoGen herd, established at the Roslin Institute by crossing purebred Charolais sires and Holstein cows in a combined F2-Backcross design, was used as resource population of this experiment. *Behaviour Testing:* A total of 196 females from the second generation (F2s and reciprocal backcrosses) were tested for the SS test. These heifers were born in three consecutive cohorts and tested at about 10-12 months of age. After separation from penmates, the test animal was recorded for five minutes and its behavioural reactions were classified as states (walking, running, gambolling, lying, kneeling, escape, standing alert, standing occupied and interacting with boards) or events (vocalise, urinate and defecate). Exploratory analysis revealed that the variation observed in this test could be condensed mainly into three measures: the sum of the durations for the walking, escaping and running states (WER), the duration of the standing alert state (SA), and the frequency of the vocalise event (V). The test was performed twice on each animal and the records obtained for each test repeat were considered as different traits in the subsequent analysis. A square-root transformation of the V records and logit transformations of the WER and SA durations were used to normalize the data for further analysis. *Genotypes and QTL analysis:* All the individuals of the population were genotyped for a total of 165 microsatellite markers distributed along the bovine autosome. Linkage maps were built for the 29 bovine autosomes. A whole genome scan based on regression analysis was performed using the QTL express software (Seaton *et al.,* 2002). Permutation testing and a Bonferroni correction were used to calculate chromosome-wide and genome-wide p-values, respectively.

**Results** Table 1 characterizes the eleven regions revealed by the regression analysis as exceeding the 5% chromosome-wide significance threshold, which were distributed on seven chromosomes. Five of the regions showed chromosome-wide p-values lower than 0.01 (designated by ▲), although none of them reached the genome-wide significance. The same region at the distal end of chromosome 19 showed evidence of linkage with SA and V on the second test repeat. The distal end of chromosome 25 was significant for V in both test repeats (V1 and V2). For six of the significant regions the additive effects were significant, whereas significant dominant effects were detected for the other five putative QTL. The proportion of the phenotypic variance explained by the QTL ranged between 3.7% (V1 on BTA25) and 6.6% (V2 on BTA25).

**Table 1** QTL identified for Social Separation test traits as exceeding the 5% chromosome-wide level

| Chr. | Trait | cM | p-value | Additive effect ± s.e.* | Dominance effect ± s.e.* |
|---|---|---|---|---|---|
| BTA 9 | V2 | 30 | 0.0171 | **-0.682 ± 0.201** | -0.268 ± 0.277 |
| BTA 15 | V2 | 47 | 0.0355 | **0.599 ± 0.192** | -0.21 ± 0.263 |
| BTA16 | WER2 | 0 | 0.0417 | -0.115 ± 0.156 | **-0.659 ± 0.213** |
| | V1▲ | 48 | 0.0058 | **0.615 ± 0.172** | -0.292 ± 0.244 |
| | SA1▲ | 87 | 0.0062 | 0.239 ± 0.130 | **0.616 ± 0.192** |
| BTA18 | V1▲ | 21 | 0.0049 | **0.584 ± 0.162** | -0.4 ± 0.24 |
| BTA19 | V2 | 72 | 0.0368 | -0.068 ± 0.191 | **0.727 ± 0.245** |
| | SA2▲ | 72 | 0.0072 | 0.057 ± 0.124 | **0.583 ± 0.159** |
| BTA25 | V1 | 28 | 0.0325 | **0.403 ± 0.159** | -0.325 ± 0.221 |
| | V2▲ | 34 | 0.0018 | **0.557 ± 0.160** | -0.311 ± 0.213 |
| BTA26 | V2 | 5 | 0.0362 | -0.185 ± 0.207 | **0.847 ± 0.302** |

* Estimates are in units of transformed data; ▲chromosome-wide p-value < 0.01

**Conclusions** Several QTL were detected for the traits appraised in the heifers under the social challenge mimicked in the SS test carried out in this experiment. Some of our results are supported by others authors' findings. For example, QTL for temperament and habituation (Schmutz *et al.,* 2001) have been mapped in a region of the bovine chromosome 9 close to the QTL for V2 here reported. Also, QTL for cattle disposition have been reported by Wegenhoft *et al.* (2005) on chromosomes 16 and 18.

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**006**

# Genome scan to detect quantitative trait loci for economically important traits in Holstein cattle using a dense SNP map

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**Introduction** Quantitative trait loci (QTL) are chromosome regions which are significantly associated with the expression of a phenotypic trait in a particular population. Detection of a QTL is carried out using association with a genetic marker, such as a single nucleotide polymorphism (SNP), which is in linkage disequilibrium (LD) with the QTL. The two main categories of association studies are linkage analyses (LA), which consider LD within families and linkage disequilibrium methods, which make use of LD across an entire population. The recent reduction in genotyping costs has allowed for testing individuals for a large number of SNP. This substantial increase in genotypic data has lead to denser marker distributions on the bovine genome thus potentially increasing the power of QTL detection studies. The objective of this study was to scan the bovine genome to detect QTL for 305 day lactation milk yield (MY), 305 day lactation fat yield (FY), 305 day lactation protein yield (PY), herd life (HL), somatic cell score (SCS), interval from calving to first service in cows (CTFS) and age at first service in heifers (AFS). HL is a measure of longevity measured in the number of lactations a cow stays in the herd. SCS refers to the amount of somatic cells a cow has in her milk and is an important indicator trait for mastitis. CTFS is the period from parturition to first insemination in days and AFS is the age in days at which a heifer was artificially inseminated for the first time. Fertility traits, such as CTFS and AFS, are indicators of reproductive efficiency.

**Materials and Methods** DNA was extracted with phenol-chloroform from the semen samples of 484 Holstein sires. These individuals were genotyped for 9919 SNP using the Affymetrix MegAllele GeneChip Bovine Mapping 10K array. The experimental design was a mixed granddaughter selective genotyping design where 427 sires were members of 10 major grandsire families. The two methods used were the variance component linkage analysis (VCLA) approach developed by George et al. (2000) and the linkage disequilibrium single locus regression method (LDRM) developed by Grapes et al. (2004). At the time of this research 4856 SNP had been physically located (in base-pairs) to chromosomes. The polymorphism information content (PIC) of the SNP was calculated and SNP with a PIC of 0.0 were excluded from both analyses. In addition, SNP with a minor allele frequency of 0.1 or less were excluded from the LDRM. The SNP centiMorgan (cM) linkage map was generated by interpolation using the microsatellite framework map available from the National Center for Biotechnology Information, Bethesda, MD, USA. In both methods estimated breeding values (EBV) were used as phenotypes and a chromosome-wide false discovery rate (FDR) threshold of 0.05 was used to account for multiple testing. Loki (Heath, 1997) was used to calculate the identical by descent probabilities and both models were computed using ASReml.

**Results** The mean number of SNP per cM and per *Bos taurus* autosome (BTA) was 1.71 and 168, respectively. The mean PIC was 0.25, with a range of 0.0 to 0.375, and skewed to the right. VCLA detected 103 potential QTL each explaining 0.07 to 0.54 of the total variance in a trait. The LDRM found 135 significant SNP associations with a range in effect of 0.14 to 0.40 EBV standard deviations (Table 1). Twelve potential QTL and 25 significant SNP associations were in close proximity to QTL cited in the literature. Both methods found significant regions on BTA 3, 5, and 16 for MY, BTA 14 and 19 for FY, BTA 1,3,16 and 28 for PY, BTA 2 and 13 for CTFS, and BTA 14 for AFS.

**Table 1**. Number of potential QTL or significant SNP associations found per trait.

| Model \ Trait | MY | FY | PY | SCS | HL | CTFS | AFS | Total |
|---|---|---|---|---|---|---|---|---|
| VCLA[1] | 15 | 6 | 53 | 4 | 0 | 20 | 5 | 103 |
| LDRM[2] | 31 | 7 | 13 | 32 | 17 | 14 | 21 | 135 |

[1]VCLA = variance component linkage analysis

[2]LDRM = linkage disequilibrium single locus regression method

**Conclusions** Both methods were effective in detecting potential QTL with dense SNP maps. The LDRM seems to detect more significant chromosome regions than VCLA for traits of lower heritability such as SCS, HL, CTFS, and AFS. The LDRM was well suited for a genome scan due to its approximately eight fold lower computational demands than VCLA. Further fine mapping could be applied on the chromosomal regions of interest found in this study.

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**007**

# Effect of beta lactoglobulin polymorphism on milk production in Holstein dairy cattle of Iran

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**Introduction** Beta-Lacto globulin (BLG) is a major protein in the milk of ruminants. Bovine BLG gene is located on the chromosome 11q28 and has two common genetic variants, 'A' and 'B' (Gene-Bank X14710). These variants are different in amino acids at positions 64 and 118, where allele 'A' has Asp and Val and allele 'B' has Gly and Ala, respectively. Difference at position 118 can be detected by PCR-RFLP (Medrano and Agiular-Cordova 1990). It is reported that allele 'A' is associated with milk production (Strzalkowska et al. 2002) and allele 'B' has a positive effect on fat percentage (Tsiaras et al. 2005). The aim of our study was to estimate the allelic frequency in polymorphic site of exon IV (allele 'A' or HaeIII (-)) of BLG gene in Holstein dairy cattle of Iran and also to find any association of total milk production (first lactation) with BLG genotypes.

**Materials and methods** Total of 55 genomic DNA samples were collected from 9 Holstein dairy farms in Iran. The DNA was extracted from whole blood (Miller *et al.* 1988). The selected primers (Medrano and Cordova 1990) were used to amplify 247 bp of exon 4. The DNA was amplified in a total volume of 25 µL containing 50 ng genomic DNA, 0.2 µM of each primer, 0.2 mM dNTP, PCR 1x buffer, 2.5mM $MgCl_2$, and 1 unit Taq DNA polymerase (GENAXXON, Germany). The amplification was carried out using 35 cycles at 94 Cº for 1min., 60 Cº for 30s and 72 Cº for 40s followed by 72 Cº for 10 min. The amplified DNA fragment was digested with 10 units *Hae*III endonuclease. Digested products were loaded on 2% agarose gel containing Ethidium-Bromide and then genotyped under UV light. Association of the total milk production and polymorphism were analyzed by JMP Software. Statistical model included the effects of herd, season, days in milk and genotypes of βLG.

Results: According to BLG sequence (Gene-Bank X14710); there are two restriction sites for *Hae*III enzyme (GG|CC) in 247-bp PCR amplified fragment. The permanent site leads to a restriction of the 247 bp product into two fragments with a length of 148 bp and 99 bp. If a polymorphic site exists, the 148 bp can be cut into two 74 bp fragments. The gene frequencies were calculated by counting method as $p = \frac{2(BB) + (AB)}{2N}$, q = 1 - p; where p is the gene frequency of allele B and q is the gene frequency of allele A. The allele frequency of 'A' was 0.29 and no homozygous genotype "AA" was found. Analysis of variance showed that the kind of genotype had no influence on milk production. The highest amount of milk production achieved in winter season. Seasons were considered Spring (21st March- 21st June), Summer (22nd June- 22nd September), autumn (24th September- 21st December), and winter (22nd December- 20th March) (Figure 1).

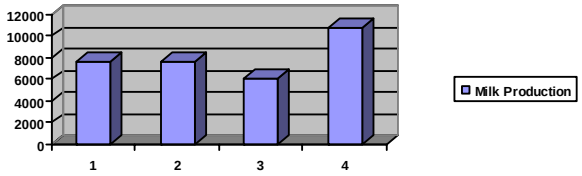


**Figure1** Effect of season on milk production (1 to 4; spring to winter, respectively)

**Conclusion**

The 'A' variant of BLG showed the lower frequency than the result of Tsiaras *et al*. (2005). No significant effect of different genotypes on milk production was observed in this study. Ojala *et al*. (1997) reported that allele 'A' has no effect on milk production, but Tsiaras *et al.* (2005) reported that heterozygous cattle have more milk production than homozygous. These conflicting reports may be due to failure of considering the family structure properly or relatedness of animals sampled.

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**008**

# Food-derived phytochemicals 'Curcumin' and 'Resveratrol' counteract the catabolic effects of inflammatory lipopolysaccharides: studies on *in vitro* models of canine and porcine arthritis

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**Introduction** Osteoarthritis (OA) and Osteochondritis Dissecans (OCD) are osteoarticular disorders that cause leg weakness, lameness, pain and suffering in companion animals, some farm animals and humans. OA is one of the most common age-related osteoarticular disorders in humans and dogs. In pigs, both OA and OCD are thought to arise from changes in the articular cartilage and growth plates within the synovial joints causing structural damage to joint tissues. Since these changes are not observed in the slow maturing wild boar, they are suggested to be a result of the modern intensive pig production industry which has very successfully selected pigs for rapid growth rates, large muscle mass and efficient feed conversion placing increased weight and mechanical stress on growth plates. The aim of this study was to establish canine and porcine articular cartilage explant models which are essentially tissue culture techniques for isolating and maintaining cartilage tissue *ex vivo* for subsequent assessment of potentially beneficial effects of specific phytonutrients. Bacterial lipopolysaccharide (LPS) was used as a catabolic mediator to create a culture model of joint inflammation mimicking the events that occur in late stages of OA and OCD. We then performed assays to determine if the dietary phytochemical 'curcumin' (derived from *Curcuma longa*) and the polyphenolic phytoalexin stilbene 'resveratrol' (found in red grapes, red wine, peanuts and some berries) are able to counteract the catabolic effects of LPS by inhibiting LPS stimulated release of cartilage matrix glycosaminoglycans (GAGs).

**Materials and methods** Samples of canine cartilage were harvested from stifle joints and the porcine cartilage from hock joints. Species specific *ex vivo* explant models were set up with the cartilage samples collected using the well established methods for the study of proteoglycan metabolism in cartilage (Carney *et al*., 1992; Takafuji *et al*., 2002). The explants were incubated in serum-free DMEM containing antibiotics and challenged with LPS at a concentration of 0.25µg/ml to mimic the joint inflammation of OA and OCD. LPS-stimulated samples and controls were co-treated with either 'curcumin' or 'resveratrol', both at 2.5µM for a period of 5 days. The tissue culture medium and the papain digested cartilage explants were analysed for GAG content in order to assess the anti-catabolic potential of 'curcumin' and 'resveratrol' using the dimethylmethylene blue (DMMB) assay for GAG release. LPS-mediated GAG release into the culture medium was then expressed as a percentage of the total GAG present in the explants prior to experimentation.

**Results** The DMMB assay confirmed the degradative effect of LPS on both canine and porcine cartilage explants. Challenge with LPS significantly increased GAG release into the culture medium. Co-treatment with 'curcumin' and 'resveratrol' antagonized the degradative effects of LPS and inhibited GAG release from the explants. GraphPad InStat software was used for statistical analysis of data (One-way ANOVA, Tukey-Kramer Multiple Comparisons Test). There was a significant increase in GAG release from LPS stimulated canine and porcine samples when compared to the control ($P<0.001$). There was a marked decrease in GAG release from the LPS stimulated canine and porcine samples with 2.5 µM 'resveratrol' when compared to untreated samples and this difference was found to be statistically significant in the canine explants ($P<0.05$). Differences were noted between the LPS stimulated samples with and without 2.5 µM curcumin but these did not reach the level of significance. The results are summarized in *Figure 1*.

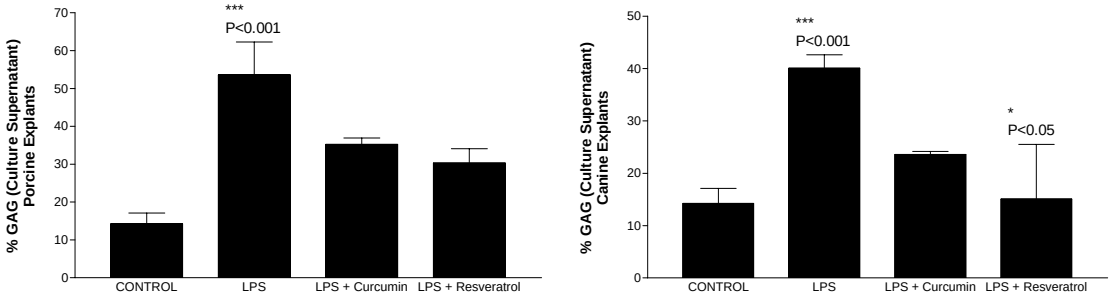


**Figure 1.** Glycosaminoglycan (GAG) release from canine and porcine cartilage explants. There was a statistically significant increase in GAG release in LPS treated explants (*** $P<0.001$).

**Conclusion** We have shown, for the first time that 'curcumin' and 'resveratrol' antagonize one of the catabolic and degradative effects of LPS, namely GAG release in explant cultures. 'Resveratrol' appeared to have a more significant anti-catabolic effect on canine explants treated with LPS. Dietary phytochemicals and phytoalexin stilbenes such as 'curcumin' and 'resveratrol' may have potential as naturally-occurring plant-derived anti-inflammatory agents, nutraceuticals and pharmacological adjuncts for the preventative treatment of OA and related osteoarticular disorders such as OCD. Future experiments will investigate the effects of these compounds on the expression and activity of inflammatory enzymes and matrix proteases in explants of canine and porcine articular cartilage.

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**009**

# The effect of age and gender on current and potential intakes of very long chain n-3 polyunsaturated fatty acids from oil-rich fish and animal derived foods in the UK

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**Introduction** The health benefits of consuming sufficient very long chain *n*-3 polyunsaturated fatty acids (VLC *n*-3 PUFA) such as EPA (C20:5) and DHA (C22:6) are well established. A key supplier of these fatty acids is oil-rich fish, however a recent study relating to UK adults indicated that only 27% of the population consume oil-rich fish and current intakes of VLC n-3 PUFA are substantially sub-optimal (Givens and Gibbs, 2006). There is also evidence to indicate that *in vivo* conversion of alpha linolenic acid (C18:3 *n*-3) to EPA and DHA is limited and highlights the need for an increased supply of preformed EPA and DHA in the diet. The role of animal-derived foods in the supply of VLC n-3 PUFA has been identified and emphasis has been placed on the need to enrich foods such as poultry meat which are widely consumed in order to make a valuable contribution to the supply of EPA and DHA to those with low or zero oil-rich fish consumption. However the effect of age and gender on intakes remained unclear. The objective of this study was to investigate the effect of age and gender on oil-rich fish consumption.

**Materials and methods** NDNS (2003) food consumption data on white, oil-rich and other fish, meat, milk and their products were used along with intakes of eggs from BEIS (2005) to calculate mean intakes of these foods for males and females in four age groups. In addition, NDNS (2003) raw data were obtained and oil-rich fish intakes were re-calculated to exclude canned tuna and percentage consumption of oil-rich fish in each group was determined. The concentrations of EPA and DHA in 'normal' and 'enriched' foods used to calculate mean current and potential intakes of EPA and DHA were as reported by Givens and Gibbs (2006).

**Results** Percentage consumers of oil-rich fish, total current mean intakes of EPA and DHA from fish and animal-derived foods and the potential contribution of 'enriched' animal-derived foods according to gender and age are given in Table 1 below. Results show that mean intakes of EPA and DHA increase with age and that oil-rich fish consumption is very low in younger age groups particularly 19-24 year olds. Interestingly, the contribution to intake from animal-derived foods is greater in males than females and thus the impact of enriching animal-derived foods may have the greatest effect on intakes in males, particularly 19-24 year olds where current mean intake of EPA and DHA from oil-rich fish is critically low. It has been found that males aged 19-34 years consume higher than average amounts of poultry meat and of the animal-derived foods with the potential to be enriched, poultry meat could be a large contributor to intakes as calculations have revealed that it could provide 86, 93, 95 and 72 mg EPA+DHA/person/day for males aged 19-24, 25-34, 35-49 and 50-64 years respectively.

**Table 1** *Estimated mean intakes of EPA and DHA*

| Gender | Age group | % | Mean intake of EPA+DHA (mg/person/d) | | |
|---|---|---|---|---|---|
| | | Consumers of oil-rich fish | Total | 'Normal' animal derived foods | 'Enriched' animal derived foods |
| Male | 19-24 | 3 | 110 | 51 | 223 |
| | 25-34 | 19 | 185 | 56 | 324 |
| | 35-49 | 29 | 262 | 56 | 275 |
| | 50-64 | 36 | 343 | 50 | 272 |
| | All males | 27 | 259 | 54 | 281 |
| Female | 19-24 | 13 | 109 | 38 | 176 |
| | 25-34 | 17 | 144 | 35 | 176 |
| | 35-49 | 25 | 213 | 41 | 205 |
| | 50-64 | 42 | 335 | 37 | 205 |
| | All females | 27 | 226 | 38 | 196 |

**Conclusions** Interpretation of food survey data is difficult; however, it is clear that current intakes of EPA and DHA among young people are estimated to be below the recommended intake of 450mg/day (SACN/COT, 2004) and therefore substantially sub-optimal. Young men are particularly vulnerable as only 3% are consumers of oil-rich fish. However, their relatively high intakes of animal-derived foods, particularly chicken, mean that intakes could be increased greatly with enriched versions in the diet. There is a need to emphasise the importance of ensuring adequate intakes of VLC *n*-3 PUFA in young people in particular as low intakes over the long-term, especially if eating habits do not change, could contribute to a significant increase in the burden of chronic disease and health costs in the future.

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**010**

# Effect of substitution of a proportion of the concentrate in grass silage/concentrate-based diets with extruded linseed on performance and meat quality of dairy bulls

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**Introduction** While meat from ruminants (beef and lamb) has been shown to have high levels of saturated and low levels of polyunsaturated fatty acids (PUFA) (Wood *et al* 1999), the beneficial properties of beef include the lower n-6:n-3 ratio, particularly under grass-fed systems, relative to pork or poultry and the presence of appreciable quantities of α linolenic acid, eicosapentaenoic and docosapentaenoic acid. The latter have been shown to reduce the incidence of cardio-vascular disease in humans. While feeding whole linseed has been shown to enhance the fatty acid composition of muscle (Scollan *et al* 2001), whole linseed is subject to significant levels of biohydrogenation in the rumen. Extruded linseed (Valomega) is promoted as increasing conjugated linoleic acid and α linolenic acid in meat through improving the efficiency of fatty acid metabolism in the rumen and intestine. The objective of the current study was to evaluate the effect of level of inclusion of extruded linseed in diets of dairy bulls on fatty acid composition of meat and instrumental meat quality.

**Material and methods** Forty eight dairy bred bulls (18 Holstein-Friesian, 6 Norwegian, 4 Norwegian X Holstein-Friesian, 12 Holstein X Norwegian and 8 Jersey X Holstein), initial live weight 350 ± 30.9 kg and 11 ± 0.7 months of age were used in the trial. The bulls were allocated to one of four dietary treatments balanced for live weight, genotype and age as follows: grass silage/concentrate-based diet (45:55 ratio on a dry matter (DM) basis) plus zero, 0.4, 0.8 or 1.2 kg of extruded linseed (Valomega 160 BCC). The concentrate consisted of (g/kg fresh) rolled barley 400, maize 230, sugar beet pulp 170, soyabean 160, molasses 30 and minerals 10 and offered daily on top of the grass silage with or without the linseed supplement. Concentrate intake was adjusted to ensure that total metabolizable energy (ME) intake from the supplement component of the diet was constant across all treatments (68 MJ/head/day). Dry matter intake was recorded daily throughout the trial. Animals were weighed on two consecutive days initially and prior to slaughter and at fortnightly intervals throughout the trial. Animals were slaughtered in three batches after 120, 147 and 185 days on experiment. Cold carcass weight, conformation, fat classification and fat deposition were measured at slaughter. A sample of the *L dorsi* muscle was subjected to instrumental meat quality analysis (Okeudo and Moss 2004) and fatty acid analysis (Amer *et al*., 1985). Data were analysed by analysis of variance (Genstat 5) with the four levels of linseed supplementation as fixed effects, pen number as blocks and genotype, age and liveweight at start of trial and days on experiment as covariates. Slaughter data were also adjusted to a constant cold carcass weight (280 kg).

**Results** Supplementation with extruded linseed had no significant effect on animal performance or carcass characteristics. In terms of fatty acid composition of the muscle, extruded linseed significantly reduced the ratio of saturated to unsaturated fatty acids ($P<0.05$) and the ratio of omega-6:omega-3 fatty acids ($P<0.001$). No other significant effects on fatty acid composition were obtained.

**Table 1** Effect of level of linseed supplementation on the performance and meat quality of beef cattle

| | Linseed supplementation (kg/day) | | | | sem | sig |
|---|---|---|---|---|---|---|
| | 0 | 0.4 | 0.8 | 1.2 | | |
| Silage dry matter intake (kg/day) | 3.7 | 4.0 | 4.0 | 3.7 | 0.19 | NS |
| Total dry matter intake (kg/day) | 8.7 | 8.9 | 8.7 | 8.3 | 0.19 | NS |
| Live weight gain (kg/day) | 1.23 | 1.34 | 1.29 | 1.27 | 0.041 | NS |
| Dressing proportion (g/kg) | 519 | 504 | 512 | 511 | 4.5 | NS |
| Marbling score† | 2.5 | 2.5 | 2.3 | 2.5 | 0.15 | NS |
| Periphrenic and retroperiteoneal fat (kg) | 16.9 | 16.8 | 18.4 | 18.1 | 1.21 | NS |
| Warner Brazler Shear Force (kg/cm$^2$) | 3.37 | 3.35 | 2.78 | 2.95 | 0.204 | NS |
| Fatty acid concentration (mg/100 g muscle) | | | | | | |
| α linolenic acid | 35.5 | 37.8 | 47.1 | 47.7 | 5.87 | NS |
| Saturated:unsaturated | 0.99$^{c}$ | 0.95$^{bc}$ | 0.91$^{ab}$ | 0.80$^{a}$ | 0.024 | * |
| Omega-6:omega-3 | 5.1$^{c}$ | 3.8$^{b}$ | 3.3$^{ab}$ | 2.8$^{a}$ | 0.23 | *** |

Means within rows with same superscripts are not significantly different ($P>0.05$); †8 point scale, 1=low marbling, 8=high marbling

**Conclusions** While substitution of a proportion of the concentrate in grass silage/concentrate-based diets offered to dairy bulls with extruded linseed had no significant effect on the individual fatty acid composition of muscle, the omega-6:omega-3 ratio was reduced to the recommended level of less than 4 (Department of Health, 1994).

**Acknowledgements**. This work is funded by DARDNI

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# Effect of dietary fish and soya oil supplementation on muscle fatty acid concentrations and oxidative lipid stability in beef cattle

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**Introduction** Some studies have shown a synergistic effect of supplementing dairy cow diets with a blend of fish and soya oil on the concentration of conjugated linoleic acid (CLA) in milk. This is apparently due to increased accumulation of ruminal and tissue concentrations of vaccenic acid (VA), the main substrate for Δ-9 desaturase catalysed, *de novo* tissue synthesis of the cis 9, trans 11 isomer of CLA. Furthermore, recent *in vitro* studies suggest that the dietary ratio of linoleic acid to the omega-3 polyunsaturated fatty acids (ω-3 PUFA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) may be an important mediator of this synergism (AbuGhazaleh and Jenkins, 2004). Additionally, dietary supplementation with ω-3 PUFA can shorten the shelf life of meat. The objectives of the current study were to examine level and duration of dietary PUFA supplementation on tissue fatty acid concentrations and on lipid oxidative stability of beef cattle.

**Materials and methods** Forty-eight continental cross young beef bulls were blocked on age, bodyweight, and breed and assigned to one of four isolipid and isonitrogenous (15% CP) dietary treatments over a 100-day finishing period. Animals were individually offered straw (10% of DMI) and barley based concentrate rations (90% of DMI) ad libitum. The concentrates contained either (i) 6% soyaoil (control; CON); (ii) 6% soyaoil + 1% fishoil (FO1); (iii) 6% soyaoil + 2% fishoil (FO2) and (iv) 8% palmitic acid (Palmit 80®) for first 50 days and 6% soyaoil + 2% fishoil (FO2) for latter 50 days (FO2(50)). Palmit 80 was added to CON and FO1 to give 8% added lipid on a DM basis to all concentrates. The soyaoil had a linoleic acid concentration of 53 per cent while the fishoil (FO) had EPA and DHA concentrations of 39 and 24 % respectively. All diets were supplemented with *dl*-α-tocopheryl acetate at 200iu $kg^{-1}$ concentrate DM. Following slaughter steaks were recovered from the *M. longissimus dorsi* (LD) and were analysed for fatty acid profile by GC. Lipid oxidative stability of cooked LD samples was measured at 0, 24, and 72 h post cooking. The data were analysed using mixed models ANOVA (PROC MIXED, SAS, 2001), with repeated measures and orthogonal contrasts used as appropriate.

**Results** Muscle concentrations of EPA and DHA were higher ($P < 0.001$) in all FO treatments compared with CON, though there was no difference within FO treatments. Concentrations of VA were higher in FO1 and FO2 compared with CON ($P < 0.01$) or FO2(50) ($P < 0.05$) but there was no difference ($P > 0.05$) between FO(50) and CON. There was no effect ($P > 0.05$) of treatment on the concentration of cis 9, trans 11 CLA. The trans 10 cis 12 CLA isomer was higher in FO2 compared with the other treatments ($P < 0.05$). The effect of treatment on the oxidative stability of lipid from cooked *L. dorsi* over time is presented in Fig. 1. There was no treatment x time interaction for malondialdehyde (MDA) concentrations in *L. dorsi* ($P > 0.05$). After 24 and 72 h post cooking, MDA concentrations in CON were lower compared with the three fishoil treatments ($P < 0.01$). Although there was no statistically significant difference between fishoil supplemented treatments at either time point ($P > 0.05$), there was a tendency towards a difference between FO2 and FO1 at both 24 and 72h ($P = 0.08$).

**Table 1** Concentration of fatty acids in *M. longissimus dorsi* (g/100g lipid)

| | CON | FO1 | FO2 | FO2(50) |
|---|---|---|---|---|
| 18:1 (VA) | $4.99^{a}$±0.432 | $7.33^{b}$±0.403 | $7.53^{b}$±0.403 | $5.66^{a}$±0.403 |
| 18:1n9c | $33.23^{a}$±0.899 | $31.05^{a,b}$±0.839 | $29.11^{b}$±0.839 | $31.31^{a,b}$±0.839 |
| 18:2n6c | $8.39^{a}$±0.612 | $6.75^{b}$±0.571 | $5.68^{b}$±0.571 | $6.43^{a,b}$±0.571 |
| CLA t10,c12 | $0.06^{a}$±0.010 | $0.05^{a}$±0.011 | $0.10^{b}$±0.011 | $0.03^{a}$±0.012 |
| CLA c9,t11 | 0.64±0.087 | 0.64±0.081 | 0.71±0.081 | 0.78±0.081 |
| 18:3n-3 | 0.47±0.041 | 0.52±0.038 | 0.54±0.038 | 0.45±0.038 |
| 20:1 | $0.17^{a}$±0.023 | $0.26^{b}$±0.022 | $0.37^{c}$±0.022 | $0.27^{b}$±0.022 |
| 20:2 | $0.07^{a}$±0.012 | $0.10^{a,b}$±0.011 | $0.14^{b}$±0.011 | $0.09^{a}$±0.011 |
| 20:3n6 | $0.26^{a}$±0.038 | $0.34^{a,b}$±0.036 | $0.47^{b}$±0.036 | $0.39^{a}$±0.036 |
| 20:4n6 | 1.05±0.136 | 0.97±0.127 | 0.89±0.127 | 1.15±0.127 |
| 20:5n3 (EPA) | $0.34^{a}$±0.184 | $1.29^{b}$±0.172 | $1.83^{b}$±0.172 | $1.45^{b}$±0.172 |
| 22:0 | 0.015±0.011 | 0.03±0.014 | 0.011±0.009 | 0.023±0.021 |
| 22:2 | $0.08^{a}$±0.134 | $0.40^{a,c}$±0.088 | $1.14^{b}$±0.088 | $0.67^{c}$±0.088 |
| 22:5n3 (DPA) | $0.43^{a}$±0.079 | $0.65^{a,b}$±0.073 | $0.80^{b}$±0.073 | $0.71^{a,b}$±0.073 |
| 22:6n3 (DHA) | $0.02^{a}$±0.050 | $0.39^{b}$±0.047 | $0.32^{b}$±0.047 | $0.35^{b}$±0.047 |

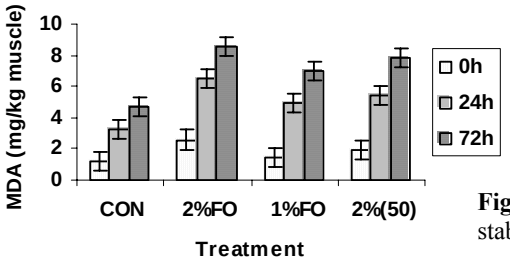


**Figure 1** Effect treatment and time post cooking on the oxidative stability of *L. dorsi*

**Conclusion** Although inclusion of fishoil increased tissue concentrations of both ω-3 PUFA and VA there was no increase in c9, t11 CLA suggesting a possible inhibitory effect of the ω-3 PUFA on Δ-9 desaturase activity. Furthermore, increasing the concentration of dietary ω-3 PUFA tended to reduce the oxidative stability of beef post cooking.

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# Effect of dietary fish and soya oil supplementation on Δ-9 desaturase relative gene expression in muscle of beef cattle

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**Introduction** Supplementation of cattle diets with a blend of oils rich in omega-3 polyunsaturated fatty acids (ω-3 PUFA) and linoleic acid have a synergistic effect on the accumulation of ruminal and tissue concentrations of vaccenic acid (VA), the main substrate for Δ-9 desaturase catalysed *de novo* tissue synthesis of the cis 9, trans 11 isomer of conjugated linoleic acid (CLA). This dietary strategy translates into increases in milk concentrations of CLA in dairy cows, however, CLA concentrations in the muscle of beef animals have not always been increased (Kenny *et al*., 2007). Studies with *in-vitro* mammary cell lines (Keating *et al*., 2006) and mice (Ntambi *et al*., 1999) suggest that ω-3 PUFA may inhibit expression of Δ-9 desaturase. Furthermore, the promoter regions of the bovine Δ-9 desaturase gene have been shown to contain a conserved PUFA response region (Keating *et al*., 2005) and this has been suggested as a mechanism through which ω-3 PUFA may modulate Δ-9 desaturase expression in bovine muscle. Currently there is no evidence to support this hypothesis. The objective of this study, therefore, was to determine the influence of ω-3 PUFA dietary supplementation on the expression of Δ-9 desaturase gene in bovine muscle.

**Materials and methods** Forty-eight continental cross young beef bulls were blocked according to liveweight and randomly allocated within block to one of four isolipid and isonitrogenous diets. Diets and feeding regime were described by Kenny *et al*., (2007). Briefly, all four diets contained 6% soyaoil, in combination with either 0 (CON), 1 (FO1), or 2 (FO2) per cent of a high ω-3 fishoil product. Animals on CON, FO1 and FO2 were fed for 100 days prior to slaughter while the fourth treatment group (FO2(50)) were offered FO2 for 50 days prior to slaughter. Palmitic acid was added to each diet as appropriate to give 8% added lipid in the DM. Samples of *M. longissimus dorsi* were harvested within 30 min. post mortem and stored at -80°C. Muscle concentrations of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), VA and CLA were measured using gas chromatography. Total RNA was isolated from fragmented frozen muscle tissue using TRIzol reagent. RNA quantity was determined by absorbance at 260 nm. RNA quality was assessed using the Agilent Bioanalyzer 2100. One microgram RNA was reverse transcribed to generate cDNA. SYBR Green quantitative real time RT-PCR reactions were performed to measure the relative expression of Δ-9 desaturase. β-actin was used as a reference gene following evaluation of a number of 'housekeeping' genes using the 'GeneNorm' software package. All amplified PCR products were sequenced to verify their identity. Gene expression results were calculated using the $2^{-\Delta\Delta CT}$ method. Data were analysed using PROC MIXED SAS, (2001). As Δ-9 desaturase gene expression has previously been related to adiposity in bovine subcutaneous tissue, the fat concentration of the muscle was used as a covariate in this analysis.

**Results** Muscle concentrations of both EPA and DHA were higher ($P < 0.001$) in all fishoil supplemented groups compared with CON. Although concentrations of VA were higher in animals receiving fishoil compared with those on CON ($P < 0.01$) there was no effect ($P > 0.05$) of treatment on the concentration of cis 9, trans 11 CLA (Kenny *et al*., 2007). Expression of mRNA for Δ-9 desaturase was decreased 2.6 ($P < 0.05$), 4.6 ($P < 0.01$) and 4.1 ($P < 0.05$) fold, respectively in FO1, FO2 and FO2(50) (Figure 1). Furthermore, there was a negative association between Δ-9 desaturase expression level and tissue concentrations of both EPA ($R^2 = 0.20$; P = 0.05) and DHA ($R^2 = 0.23$; P = 0.04).

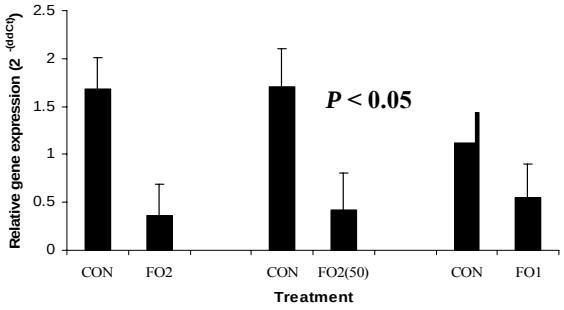


**Figure 1** Effect of treatment on Δ-9 desaturase mRNA relative gene expression analyses of muscle tissue.

**Conclusions** The results of this study show for the first time that dietary ω-3 PUFA supplementation inhibits gene expression of Δ-9 desaturase in the muscle of beef cattle. These findings have implications, therefore, for the proper design of dietary strategies aimed at increasing both the CLA and ω-3 PUFA concentration of beef.

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**013**

# The composition and oxidative stability of lipids in longissimus muscle from grazing cattle supplemented with sunflower oil alone or with fishoil

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**Introduction** Compared with conventional indoor rations, consumption of grazed grass by cattle improved the fatty acid profile of beef, by increasing the polyunsaturated fatty acid (PUFA) and conjugated linoleic acid (CLA) concentrations and decreasing the n-6:n-3 PUFA ratio (French et al., 2000). Supplementation of grazing cattle with sunflower oil (S), increased the concentration of CLA in muscle but also increased the n-6: n-3 PUFA ratio (Noci et al., 2006). Supplementation with fishoil (F), a source of long-chain n-3 PUFA might decrease this ratio while enhancing CLA synthesis. However, increasing the concentration of PUFA, particularly the long-chain PUFA, may predispose muscle lipids and pigments to oxidation, with consequent impairment of lipid and colour stability. The objectives were to determine the effect of supplementation of grazing cattle with S and F on (i) tissue n-3 PUFA and CLA concentrations and (ii) the colour and lipid stability of beef.

**Materials and methods** Grazing Charolais crossbred heifers (initial bodyweight = 407 kg, s.d. 31.3) were offered (n = 12/treatment): grazing only (G), or a daily supplement of 2.5 kg concentrates that supplied 290g S (S1), 415g S (S2), 290g S + 85g F (FS1) or 415g S + 85g F (FS2). Animals were restrained in an auto-locking feeding trailer and offered the supplements on an individual basis. Animals were slaughtered after 150 days and lipids from the longissimus muscle (LM) were separated into neutral (N) and polar (P) fractions, methylated and analysed by gas chromatography. Samples of thawed LM were placed in retail display trays and stored under an atmosphere of 80% $O_2$ : 20% $CO_2$. Colour (redness) and lipid oxidation (2-thiobarbituric acid reactive substances; TBARS) were measured at 3-day intervals during retail display. Data were analysed according to a randomised block design using Genstat 6.0. "A priori" contrasts were used to test for effects of oil supplementation, level of S in the diet and F inclusion.

**Results** Data are summarised in Table 1. Daily bodyweight gain and carcass weight averaged 834g and 294kg, respectively, and did not differ (P>0.05) between treatments. Supplementation *per se* increased the proportion of CLA, vaccenic acid (trans-11 18:1; VA) and the n-6: n-3 PUFA ratio in both N and P lipid fractions. Increasing S consumption had no effect on the fatty acids presented in Table 1. Inclusion of F in an S-based supplement increased the proportion of CLA and VA and decreased the n-6 :n-3 PUFA ratio in both N and P lipid fractions. There were no treatment effects on either lipid oxidation or colour stability (data not shown) of muscle.

**Table 1** Concentration of fatty acids (mg/100g fatty acids), and TBARS (mg malondialdehyde/kg muscle) in *M. longissimus dorsi*

| Treatment | G | S1 | S2 | FS1 | FS2 | s.e.m. | Oil | S | F |
|---|---|---|---|---|---|---|---|---|---|
| Neutral lipids | | | | | | | | | |
| CLA c9t11 | 1.29 | 1.80 | 2.18 | 2.32 | 2.44 | 0.132 | * | NS | * |
| VA | 2.78 | 4.40 | 4.96 | 5.51 | 5.33 | 0.318 | * | NS | * |
| n-6:n-3 Ratio | 1.64 | 3.53 | 3.33 | 2.55 | 2.83 | 0.364 | * | NS | * |
| Polar lipids | | | | | | | | | |
| CLA c9t11 | 0.59 | 0.88 | 1.12 | 1.35 | 1.20 | 0.101 | * | NS | * |
| VA | 1.10 | 3.19 | 3.77 | 5.64 | 4.46 | 0.529 | * | NS | * |
| n-6:n-3 Ratio | 1.81 | 3.94 | 4.24 | 2.32 | 2.54 | 0.557 | * | NS | * |
| TBARS | | | | | | | | | |
| - Day 4 | 3.9 | 3.4 | 4.1 | 3.8 | 3.6 | 0.29 | NS | NS | NS |
| - Day 7 | 5.4 | 5.5 | 6.0 | 5.3 | 5.5 | 0.30 | NS | NS | NS |
| - Day 14 | 8.4 | 7.9 | 8.4 | 8.5 | 7.9 | 0.30 | NS | NS | NS |

**Conclusions** Inclusion of fishoil in a sunflower oil-based supplement for grazing cattle augmented the increase in CLA while altering the n-6: n-3 PUFA ratio in a desirable direction. These modifications in fatty acid composition did not compromise the shelf-life of muscle.

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**014**

# The fatty acid composition of muscle fat in Charolais steers: influence of grass versus concentrate feeding

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**Introduction** Nutritional approaches are the most important strategy for altering the fatty acid composition of muscle lipids (Scollan *et al*., 2006). Grass relative to concentrate feeding increases the content of *n*-3 polyunsaturated fatty acids (PUFA) resulting in a low *n*-6:*n*-3 PUFA ratio. Ruminally protected plant lipids enhance PUFA content very significantly resulting in beneficial P:S and *n*-6:*n*-3 ratios (Scollan *et al*., 2006). This study considered the effects of finishing steers (1) outdoors on grass ± concentrate versus (2) indoors on straw/concentrate ± a protected lipid supplement with one of two levels of vitamin E on the fatty acid composition of the *m*. *longissimus thoracis et lumborum*.

**Materials and methods** Forty eight Charolais steers (initial live weight 506 kg (s.e.d. 4.7)) were randomly allocated to one of six dietary treatments (each consisting of eight animals) (1) *ad libitum* grazed perennial ryegrass (G) (2) grazed ryegrass + 2.5 kg concentrate (GC1), (3) grazed ryegrass + 5.0 kg concentrate (GC2), (4) straw plus concentrate (control); (5) straw + concentrate (standard vitamin E, 25 mg/kg)+ 600 g/d protected lipid supplement (PLS; *n*-6:*n*-3 ratio of 1:1) (PLSV1) and (6) straw + concentrate (high vitamin E, 500 mg/kg DM) + 600 g/d PLS (PLSV2). Straw was offered *ad libitum*. The grazed animals were maintained on rotational grazed paddocks while the straw/concentrate treatments (4, 5 & 6) were kept indoors. All animals were fed to achieve a similar rate of carcass gain by either restricting grass (treatments 2, 3) or restricting intake of the concentrate. The concentrate was based on barley, molasses, sugarbeet pulp, megalac and premix. Animals were slaughtered after 100 days on treatment and samples of *m*. *longissimus thoracis et lumborum* (LTL) were taken at 48h post-mortem for fatty acid analysis. An ANOVA with diet as the main factor was used to analyse the data.

**Results** Half carcass weights were higher for the outdoor relative to indoor animals ($P < 0.001$), but carcass fatness was similar. Total muscle fatty acids were similar across treatments (Table 1). Increasing concentrate outdoors (GC1 and 2) resulted in higher amounts of 18:2*n*-6 and lower 18:3*n*-3 relative to grass only. There was evidence that EPA was also reduced (GC2 *v*. G). Indoors, inclusion of the PLS resulted in large increases in 18:2*n*-6 and 18:3*n*-3, which did not impact on the longer chain C20 PUFA. PLS resulted in a large increase in P:S ratios (i.e PLSV1 *v*. control). Grass feeding resulted in the lowest *n*-6:*n*-3 ratios.

**Table 1** Fatty acid content (mg/100g muscle) of *longissimus thoracis et lumborum*

| | Outdoor | | | Indoor | | | | |
|---|---|---|---|---|---|---|---|---|
| | Grass (G) | GC1 | GC2 | Control | PLSV1 | PLSV2 | SED | P |
| Half carc. wt (kg) | 161.5 | 164.6 | 166.6 | 157.0 | 156.8 | 156.0 | 2.42 | 0.001 |
| | Fatty acid composition (mg/100 g muscle) | | | | | | | |
| Total fatty acids | 1725 | 1800 | 1637 | 1778 | 1880 | 1638 | 274.5 | NS |
| 16:0 | 405 | 432 | 383 | 435 | 456 | 383 | 73.7 | NS |
| 18:0 | 254 | 256 | 225 | 266 | 260 | 234 | 43.1 | NS |
| 18:1*n*-9 | 567 | 609 | 520 | 546 | 528 | 440 | 100.2 | NS |
| 18:1 *trans* | 33.0 | 31.0 | 32.5 | 26.5 | 29.6 | 28.0 | 7.01 | NS |
| CLA | 8.5 | 8.2 | 9.1 | 6.8 | 8.0 | 7.6 | 2.12 | NS |
| 18:2*n*-6 | 58.4 | 64.6 | 77.5 | 91.2 | 178.3 | 171.8 | 10.01 | 0.001 |
| 18:3*n*-3 | 27.4 | 21.3 | 18.6 | 14.7 | 42.2 | 40.5 | 3.44 | 0.001 |
| EPA | 14.5 | 13.2 | 12.4 | 11.3 | 11.9 | 10.8 | 1.18 | 0.039 |
| DHA | 1.9 | 2.6 | 2.2 | 1.8 | 1.7 | 1.7 | 0.28 | 0.017 |
| DPA | 19.1 | 19.7 | 18.8 | 16.9 | 13.5 | 13.2 | 1.21 | 0.001 |
| P:S | 0.14 | 0.13 | 0.16 | 0.15 | 0.30 | 0.35 | 0.027 | 0.001 |
| *n*-6:*n*-3 | 1.44 | 1.83 | 2.29 | 2.96 | 3.10 | 3.12 | 0.223 | 0.001 |

**Conclusions** Total fatty acids were relatively low which contributed to higher P:S ratios across all treatments due to acknowledged relationship between total lipid and P:S (Scollan *et al*., 2006). Grass feeding resulted in higher levels on *n*-3 PUFA resulting in very favourable *n*-6:*n*-3 ratios. However, these beneficial effects were diluted by feeding additional concentrate outdoors. Protected lipids resulted in large increases in 18:2*n*-6 and 18:3*n*-3 contributing to a very beneficial P:S ratio.

**Acknowledgements** This work was supported by Department for Environment Food and Rural Affairs.

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**015**

# Effect of the physical form of a lipid supplement on the polyunsaturated fatty acids, including conjugated linoleic acid, in muscle tissues of red deer and sheep

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**Introduction** Proportions of polyunsaturated fatty acid (PUFA) in ruminant tissues are generally low as dietary PUFA including linoleic acid (LA) and α-linolenic acid (ALA) undergo biohydrogenation to stearic acid in the rumen. Conjugated linoleic acid (CLA) is formed during biohydrogenation as an intermediate metabolite. Feeding PUFA-rich sources can enhance deposition in the tissues of ruminants (Bolte *et al*, 2002), but the degree of lipid hydrogenation may depend on the source form and the ruminant species (Rowell-Schäffer *et al*, 2001). Bolte *et al*, (2002) showed that oilseed-fed sheep had higher PUFA proportions in their tissues than sheep fed a beet pulp-based diet but Rowell-Schäffer *et al* (2001) found in deer tissues higher PUFA proportions than in those of sheep. Possible effects of the form of the lipid source and the ruminant species on the fatty acids deposited were tested.

**Material and methods** Twenty-four red deer yearling stags and 24 castrated male sheep (initial liveweights: 90.2±2.14 and 26.8±0.31kg, respectively) were allocated to three treatments: Red deer 1) DECO 1.2kg high-quality grass hay (110gCP and 9.5MJ ME/kg DM)+0.6kg concentrate supplement (160gCP and 11MJ ME/kg DM); 2) DESM: 1.2kg high-quality grass hay+0.6kg concentrate supplement+182g whole sunflower seed meal; and 3) DESO: 1.2kg high-quality grass hay+0.6kg concentrate supplement+66g sunflower seed oil ; sheep 1) SHCO: 0.35kg high-quality grass hay+0.175kg concentrate supplement; 2) SHSM: 0.35kg high-quality grass hay+0.175kg concentrate supplement+91g whole sunflower seed meal; and 3) SHSO: 0.35kg high quality grass hay+0.175kg concentrate supplement+33g sunflower seed oil. All sunflower seed treatments provided the same amount of oil. The treatments were imposed for nine weeks. All animals were kept indoors in separate pens and fed individually. At slaughter the warm carcasses were sampled from four sites (flank, loin, rump and shoulder). Lipids were extracted from samples and FAME prepared and analysed by GC. Statistical analysis was undertaken using a Linear Mixed Model in GenStat. To avoid inter-dependence with the other fatty acids (FA), the content of individual FA in tissue samples was expressed as a ratio to the concentration of stearic acid ($C_{18:0}$) in the samples.

**Results** Muscle tissues in red deer had a higher ratio ($P<0.001$) of LA and ALA than did those of sheep, but muscle tissues of unsupplemented sheep had a higher ratio ($P<0.05$) of CLA than unsupplemented red deer. Although sunflower seed treatments had no effect on the ratio of ALA in muscle tissues of sheep, sunflower seed oil significantly increased the ratio of LA. In contrast, sunflower seed oil reduced significantly the ratio of LA in the muscle tissues of red deer (Table 1). There was a decrease in the ratio of ALA in the muscle tissues of red deer when sunflower seed meal was fed as a supplement ($P<0.001$) and even more pronounced if sunflower seed oil was given. Sunflower seed oil supplementation, however, increased the ratio of CLA in the muscle tissues of both red deer and sheep (Table 1).

**Table 1** Effect of treatments on the individual fatty acid ratio in muscle tissues of red deer and sheep[†]

| | Red deer | | | Sheep | | | |
|---|---|---|---|---|---|---|---|
| Fatty acid | DECO | DESO | DESM | SHCO | SHSO | SHSM | SEM |
| 16:0$^{***}$ | 1.349$^{a}$ | 1.405$^{a}$ | 1.449$^{a}$ | 0.943$^{b}$ | 0.936$^{b}$ | 0.777$^{b}$ | 0.1130 |
| 16:1$^{***}$ | 0.400$^{a}$ | 0.466$^{a}$ | 0.615$^{a}$ | 0.046$^{b}$ | 0.038$^{b}$ | 0.033$^{b}$ | 0.0904 |
| *t*11 18:1$^{**}$ | 0.093$^{a}$ | 0.240$^{b}$ | 0.120$^{a}$ | 0.145$^{c}$ | 0.298$^{d}$ | 0.155$^{c}$ | 0.0216 |
| c9 18:1$^{***}$ | 0.922$^{a}$ | 0.982$^{a}$ | 1.039$^{a}$ | 1.460$^{b}$ | 1.316$^{b}$ | 1.284$^{b}$ | 0.0720 |
| ALA$^{‡***}$ | 0.219$^{a}$ | 0.131$^{b}$ | 0.171$^{c}$ | 0.046$^{d}$ | 0.038$^{d}$ | 0.039$^{d}$ | 0.0069 |
| LA$^{§***}$ | 1.292$^{a}$ | 1.150$^{b}$ | 1.285$^{a}$ | 0.137$^{c}$ | 0.275$^{d}$ | 0.165$^{cd}$ | 0.0611 |
| CLA$^{‖*}$ | 0.026$^{a}$ | 0.063$^{b}$ | 0.033$^{ac}$ | 0.046$^{d}$ | 0.065$^{b}$ | 0.045$^{cd}$ | 0.0062 |

Values in the same row with different superscripts denote significance ($^{*}P<0.05$; $^{**}P<0.01$; $^{**}P<0.001$). [†]Fatty acid ratio:- the content of individual fatty acids (mg/g) divided by the content of stearic acid (mg/g). [‡]α-linolenic acid. [§]Linoleic acid. [‖]Conjugated linoleic acid. Data presented of mean values for the four tissues analysed.

**Conclusion** Differences in the ratio of PUFA in the muscle tissues of red deer and sheep across different treatments suggested an interaction between the physical form of the lipid supplement and morphophysiological differences between these ruminant species. For example, the supply of dietary lipid within the matrix of sunflower meal may have limited accessibility of fatty acids to rumen microbes thus reducing biohydrogenation. In addition, the higher ratio of PUFA found in muscle tissues of deer may result from the escape of some of the PUFA unaltered due to greater outflow rates (see Rowell-Schäffer *et al*, 2001). The present study showed that appropriate supplementation with different sources of sunflower seed can alter the proportions of PUFA in muscle tissues of ruminants and thus their meat quality.

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**016**

# Effect of dietary source of very long chain n-3 polyunsaturated fatty acids on their concentrations in the edible tissues of the chicken

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**Introduction** The benefits to human health from consuming the very long chain *n*-3 polyunsaturated fatty acids (VLC *n*-3 PUFA) 20:5 (EPA) and 22:6 (DHA) are well known. In the human diet, oil-rich fish is a key source of VLC *n*-3 PUFA but fish consumption is so low that VLC n-3 PUFA intake is well below the minimum recommended. Other vehicles for increasing dietary supply have been explored and chicken meat is a potentially important contributor to human intakes if it is enriched with VLC n-3 PUFA, as it is responsive to dietary manipulation and is widely consumed. Enrichment of chicken meat can be achieved by supplementing the diets of growing birds with fish oil. However, reliance upon fish oil for this purpose is potentially unsustainable and the meat that is produced is more susceptible to constraints associated with its oxidative stability and organoleptic properties. Alternative sources of these fatty acids include marine algae (the primary producers of VLC *n*-3 PUFA) and fish oil encapsulated in a gelatin capsule. Both of these alternatives may increase the VLC n-3 PUFA content of the meat and also enhance its oxidative stability compared with meat that has been enriched by the inclusion of fish oil in the broiler diet. The objective of this study was to compare different sources of VLC n-3 PUFA in the broiler diet in relation to their effects on EPA and DHA concentration of the white and dark meat of the chicken.

**Materials and methods** Day old, mixed sex, Ross 308 chicks (96) were reared together as a single group for three weeks on a proprietary chick crumb. At three weeks of age the birds were randomly allocated to one of four experimental groups. Six birds were allocated to a cage and there were four cages per diet. The birds were fed diets supplemented with either soya oil (control, CON), fish oil (FFO), encapsulated fish oil (EFO) or freeze dried marine algae (ALG). The FFO, EFO and ALG diets were formulated to contain 5 g DHA/kg of feed. At six weeks of age the birds were humanely slaughtered. Samples of skinless white and dark meat were taken from two of the birds from each cage. The samples were homogenised and frozen prior to analysis. Diets and tissues were analysed for fatty acids by gas chromatography- mass spectrometry. The effects of diet, tissue and diet x tissue interaction on the concentration of VLC n-3 PUFA were determined by ANOVA.

**Results** The diets containing fish oil were much richer sources of EPA than was the diet containing algae. Concentrations (mg/kg fresh weight) of EPA were 0, 3828, 1258 and 132 and of DHA 0, 5060, 5040, and 4972 for CON, FFO, EFO and ALG respectively. The effect of treatment on the concentrations of EPA and DHA in the meat are summarised in Table 1. There were significant interactions between diet and tissue in terms of the concentration of EPA, with the increased concentration of dietary VLC n-3 PUFA having a much more marked effect on the dark meat than the white meat. Dark meat had a significantly higher concentration of EPA than white meat. The concentration of EPA was much greater in the meat of birds fed FFO and EFO compared with CON and ALG, while the concentration of DHA was greater in the meat of birds fed FFO, EFO and ALG compared with those fed CON.

**Table 1** *Effect of diet and tissue on the concentrations (mg/100 g fresh tissue) of EPA and DHA in the white and dark meat.*

| Fatty | Tissue | Diet | | | | SEM | P | | |
|---|---|---|---|---|---|---|---|---|---|
| acid | | CON | FFO | EFO | ALG | | Diet (D) | Tissue (T) | DxT |
| EPA | White | 4.1 | 30.6 | 18.4 | 6.1 | | | | |
| | Dark | 4.0 | 52.5 | 57.7 | 10.0 | 5.33 | <0.001 | <0.001 | 0.002 |
| DHA | White | 24.2 | 129 | 122 | 147 | | | | |
| | Dark | 7.9 | 120 | 139 | 93 | 12.97 | <0.001 | 0.098 | 0.063 |

**Conclusions** The higher concentrations of EPA in the meat of birds fed FFO and EFO is a reflection of the higher concentrations of EPA in the diets containing fish oil. There was no evidence of any difference between dietary DHA sources in the efficiency with which this DHA was incorporated into edible tissue. If the availability of fish oil began to decrease, the use of marine algae could become a valuable source of DHA and these data suggest it would be transferred to the edible tissues as efficiently as the DHA from fresh fish oil.

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**017**

# Effect of dietary source of very long chain n-3 polyunsaturated fatty acids in poultry diets on the oxidative stability of chicken meat

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**Introduction** Enriching chicken meat with the very long chain n-3 polyunsaturated fatty acids (VLC n-3 PUFA) 20:5 (EPA) and 22:6 (DHA) is a possible means of increasing the human consumption of these essential fatty acids as current levels of intake of these fatty acids are extremely low. However, a potential drawback of increasing the VLC n-3 PUFA content of chicken meat is that the oxidative stability of the meat is reduced. Chicken meat is enriched with VLC n-3 PUFA by the addition of fish oil to the chickens' diet. It is possible that using alternative dietary sources of VLC n-3 PUFA may increase the oxidative stability of the meat (Mooney et al., 1998). The objective of this experiment was to determine what the source of VLC n-3 PUFA in broilers' diets had on the oxidative stability of their edible tissues.

**Methods** Day old, mixed sex, Ross 308 (72) chicks were reared together as a single group for three weeks on a proprietary chick crumb, before being randomly allocated to one of six experimental groups. Six birds were allocated to a cage and there were two cages per diet. The birds were fed diets supplemented with either soya oil (control, CON), fish oil (FFO), encapsulated fish oil (EFO) or freeze dried marine algae, fed at a rate of 11 (LAG), 22 (MAG or 33 (HAG) g/t diet. The FFO, EFO and MAG diets were formulated to contain 5 g DHA/kg of feed, whereas the concentration of DHA in LAG and HAG was 2.5 and 7.5 g/kg respectively. All diets contained 10 iu Vitamin E/kg. Birds were humanely slaughtered at 42 d. Samples of skinless white and dark meat were taken from one bird in each cage. Samples were cooked ($180^0$C, 30 min, wrapped in aluminium foil), minced and analysed for volatile aldehydes by gas chromatography-mass spectrometry. The effects of diet, tissue and the interaction between diet and tissue were analysed by analysis of variance using Mintab.

**Results** The results are summarised in Table 1. The most concentrated aldehyde was hexanal. Aldehydes were usually found in greatest concentration in the dark meat, except for hexadecanal which was more abundant in the white meat. Hexadecanal was also more concentrated in the meat from birds fed CON, whereas the other aldehydes were more concentrated in the meat of birds fed the n-3 fatty acids. Birds fed FFO had lower concentrations of aldehydes in their meat than did birds fed EFO. Birds fed algae had higher aldehyde concentrations in their meat than did birds fed FFO, except for hexadecanal. The highest concentration of 2-nonenal was observed in the white meat of birds fed algae, but in the dark meat of birds fed EFO. The high concentration of hexadecanal in CON-fed birds was more evident in the white meat than the dark meat.

**Table 1** Effect of diet and tissue on the concentration (mg/kg fresh tissue) of aldehydes in cooked chicken meat

| Meat | Diet | Aldehyde | | | |
|---|---|---|---|---|---|
| | | Hexanal | 2-Nonenal | 2,4-Decadienal | Hexadecanal |
| White | CON | 0.875 | 0.035 | 0.045 | 2.97 |
| | FFO | 0.970 | 0.045 | 0.035 | 0.785 |
| | EFO | 1.56 | 0.050 | 0.140 | 0.660 |
| | LAG | 2.25 | 0.060 | 0.100 | 0.550 |
| | MAG | 2.15 | 0.070 | 0.165 | 0.380 |
| | HAG | 2.06 | 0.060 | 0.080 | 1.70 |
| Dark | CON | 2.56 | 0.070 | 0.255 | 0.745 |
| | FFO | 1.42 | 0.065 | 0.170 | 0.565 |
| | EFO | 5.07 | 0.220 | 1.240 | 0.355 |
| | LAG | 4.12 | 0.125 | 0.455 | 0.220 |
| | MAG | 4.22 | 0.125 | 0.375 | 0.205 |
| | HAG | 5.31 | 0.190 | 0.715 | 0.645 |
| SEM | | 0.4478 | 0.0161 | 0.1249 | 0.2037 |
| *P* | Diet (D) | 0.002 | 0.008 | 0.014 | <0.001 |
| | Tissue (T) | <0.001 | <0.001 | <0.001 | <0.001 |
| | DxT | 0.194 | 0.032 | 0.071 | <0.001 |

**Conclusions** The higher lipid content of dark meat compared with white meat is reflected in its lower oxidative stability. Hexadecanal production was more associated with the n-6 fatty acids found in CON's soya oil rather than the n-3 fatty acids of the other diets. The oxidative stability of the meat appeared to be reduced when birds were fed marine algae rather than fresh fish oil.

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**018**

# Relationship between the unsaturated fatty acid profile of poultry meat and the volatile aldehydes produced by the meat.

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**Introduction** Enriching chicken meat with the very long chain n-3 polyunsaturated fatty acids (VLC n-3 PUFA) 20:5 (EPA) and 22:6 (DHA) is a possible means of increasing the human consumption of these essential fatty acids as current levels of intake of these fatty acids are extremely low. However, a potential drawback of increasing the VLC n-3 PUFA content of chicken meat is that the oxidative stability of the meat is reduced. PUFA are more oxidatively unstable than monounsaturated or saturated fatty acids, and the aldehydes produced by the n-3 PUFA during autoxidation have a lower taste threshold, and are much more unpalatable, than the aldehydes associated with autoxidation of the n-6 series of PUFA. The objective of this study was to determine what relationship there was between the fatty acid profiles of chicken meat that had been enriched (by dietary means) with VLC n-3 PUFA and the volatile aldehydes that were produced by the meat after it had been cooked.

**Methods** Day old, mixed sex, Ross 308 (144) chicks were reared together as a single group for three weeks on a proprietary chick crumb, before being randomly allocated to one of six experimental groups. Six birds were allocated to a cage and there were four cages per diet. The birds were fed diets supplemented with either soya oil (control, CON), fish oil (FFO), encapsulated fish oil (EFO) or freeze dried marine algae, fed at a rate of 11 (LAG), 22 (MAG or 33 (HAG) g/t diet. The FFO, EFO and MAG diets were formulated to contain 5 g DHA/kg of feed, whereas the concentration of DHA in LAG and HAG was 2.5 and 7.5 g/kg respectively. All diets contained 10 iu Vitamin E/kg. Birds were humanely slaughtered at 42 d. Samples of skinless white and dark meat were taken from three birds in each cage. The meat from two birds was analysed for fatty acids, while the meat from the third bird (from two cages per treatment) was cooked ($180^0$C, 30 min, wrapped in aluminium foil), minced and analysed for volatile aldehydes. All analysis was done by gas chromatography-mass spectrometry. Stepwise regression was used to relate the concentrations of monounsaturated fatty acids (MUFA, 18:1), PUFA (Σ18:2, 18:3, 20:4, 20:5, 22:5, 22:6), n-3 PUFA (Σ18:3 n-3, 20:5 n-3, 22:5 n-3, 22:6 n-3), n-6 PUFA (Σ18:2 n-6, 20:4 n-6), VLC n-3 PUFA (Σ20:5, 22:5, 22:6) and VLC n-6 PUFA (20:4) to the concentrations of hexanal, 2-nonenal, 2,4-decadienal and hexadecanal..

**Results** The results are summarised in Table 1. No regression was improved by the inclusion of a second term. The most concentrated aldehyde detected was hexanal, which was associated with PUFA, and did not distinguish between n-3 and n-6 PUFA. Hexadecanal, while only weakly related to total PUFA content, was also not related more closely to n-3 PUFA content than the n-6 series of PUFA. However, 2-nonenal and 2,4 decadienal were associated with n-3 PUFA content. The predictor selected in this case was n-3 PUFA (including 18:3) and not the VLC n-3 PUFA content, which excluded 18:3 but included EPA and DHA.

**Table 1** Relationship between the concentration of fatty acids and aldehydes in chicken meat.

| Aldehyde | Regression characteristics | | | | | |
|---|---|---|---|---|---|---|
| | Constant | Coefficient | Variable | $R^2$ | P | s |
| Hexanal | 1.112 | 0.0054 | PUFA | 0.441 | <0.001 | 1.27 |
| 2-Nonenal | 0.0416 | 0.007 | n-3 PUFA | 0.450 | <0.001 | 0.048 |
| 2,4-decadienal | 0.135 | 0.012 | n-3 PUFA | 0.432 | <0.001 | 0.903 |
| Hexadecanal | 1.300 | -0.002 | PUFA | 0.168 | 0.047 | 0.763 |

**Conclusions** Enriching poultry meat with PUFA decreases its oxidative stability, and enrichment of poultry meat with n-3 PUFA will increase the production of the aldehydes 2-nonenal and 2,4-decadienal. However, this is likely to occur whether the chicken is fed a diet supplemented with 18:3, or the VLC n-3 PUFA EPA and DHA. Supplementation of this kind, to increase the human consumption of n-3 PUFA, particularly EPA and DHA, must be done with care, with the adequate use of antioxidants such as Vitamin E, to ensure that the oxidative stability and organoleptic properties of the meat are maintained.

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# Effect of dietary alpha-linolenic acid and linoleic acid level on growth performance, carcass and meat characteristics of Thai indigenous growing pigs

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**Introduction** Presently, indigenous pig farming is relatively growing in Thailand, because Thai consumers increasingly prefer the meat of indigenous pigs. Generally, indigenous pigs tend to store more fat in their carcass than pigs of commercial breeds. The nature of the dietary fat source may have an effect on fat deposition processes in indigenous pigs and subsequently on carcass and meat characteristics. For example, alpha-linolenic acid (ALA, C18:3, n-3) is an essential polyunsaturated fatty acid that is more preferentially oxidized in the body than linoleic acid (LA, C18:2, n-6) and other fatty acids. Therefore, the supplementation of ALA in pig diets should have a more beneficial effect on animal performance than LA supplementation. However, some adverse effects, e.g. meat characteristics, might result from a higher level of ALA supplementation as well. Thus, the aim of this study was to specify the effect of dietary supplementation of ALA versus LA on growth performance, carcass and meat characteristics of pigs.

**Materials and methods** Eighteen Thai indigenous pigs at 8 weeks of age were randomly allotted to two experimental groups (4 male and 5 female per group) and kept in individual metabolism cages. Experimental diets (high and low ALA) were similar, except for the inclusion level of linseed and soybean oil (Table 1). The pigs were fed either a low ALA or a high ALA diet during 5 weeks (*ad libitum* feeding and water supply). Feed intake and body weight were recorded. All pigs were slaughtered at the end of the study after which carcass and meat characteristics were determined. Hot carcass weight (exclusive internal organs), back fat thickness, and main internal organs (heart, liver, spleen, and kidney) were weighed. The right *m. longissimus dorsi* was removed to measure drip loss (%), meat pH at 45 min ($pH_1$) and at 24 hr ($pH_U$) after slaughtering, and intramuscular fat (IMF, %). Significant differences between treatment means were tested by the Student's *t* test with a 5% level of probability.

**Results** Data are given in Table 2. Supplementation of ALA to growing pigs had no significant effect on their growth performance. Carcass and meat characteristics were also not affected by ALA supplementation, although back fat thickness tended to be less in pigs fed the high ALA diet ($p = 0.112$).

**Table 1** Ingredients and chemical compositions of the experimental diets

| | Low ALA | High ALA |
|---|---|---|
| Ingredients (%) | | |
| Cassava chip | 46.53 | 46.53 |
| Soybean meal (40% CP) | 40.00 | 40.00 |
| Molasses | 4.00 | 4.00 |
| Calcium carbonate | 1.00 | 1.00 |
| Dicalcuim phosphate | 2.70 | 2.70 |
| NaCl | 0.35 | 0.35 |
| DL-Methionine | 0.17 | 0.17 |
| Premix | 0.25 | 0.25 |
| Linseed oil | 0.50 | 4.50 |
| Soybean oil | 4.50 | 0.50 |
| ***Calculated chemical compositions*** | | |
| ME (kJ/kg) | 13,643.15 | 13,623.27 |
| Protein (%) | 16.88 | 16.88 |
| Fiber (%) | 4.38 | 4.38 |
| Fat (%) | 6.04 | 6.04 |
| *% of total fat* | | |
| Alpha-linolenic acid | 0.61 | 2.44 |
| Linoleic acid | 2.48 | 0.84 |

**Table 2** Effect of dietary ALA level on growth performance, carcass and meat characteristics

| | Low ALA | High ALA |
|---|---|---|
| ***Growth performance*** | | |
| Initial live BW (kg) | 10.67 | 10.87 |
| Final live BW (kg) | 20.07 | 21.08 |
| ADG (g/d) | 311.76 | 332.35 |
| Feed:Gain (g/g) | 2.54 | 2.42 |
| ADFI (g/d) | 766.27 | 761.08 |
| ***Meat characteristics*** | | |
| Drip loss (%) | 1.62 | 1.77 |
| $PH_1$ | 6.09 | 6.21 |
| $pH_U$ | 6.03 | 6.16 |
| IMF (%) | 4.68 | 4.48 |
| ***Carcass characteristics*** | | |
| Hot carcass wt. (kg) | 15.81 | 15.96 |
| BF (cm) | 1.46 | 1.25 |
| ***Organ weight*** | | |
| Heart (g) | 92.9 | 104.9 |
| Liver (g) | 719.8 | 815.2 |
| Spleen (g) | 73.3 | 79.1 |
| Kidney (g) | 124.5 | 133.9 |

**Conclusion** The results of the current study indicate that dietary ALA supplementation has no adverse effects on growth performance, meat and carcass characteristics of young indigenous pigs. Back fat thickness tended to be reduced in pigs fed high ALA diets, indicating that ALA might reduce fat deposition in indigenous pigs.

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# Effects of fatty acid sources on conjugated linoleic acid (CLA) and other fatty acids in dairy milk

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**Introduction** Conjugated linoleic acid (CLA) is aniticarcinogenic, antiatherogenic and antidiabetogenic actives. Research has therefore focused on methods of increasing CLA content in milk fat. Amount of CLA in milk fat was highly related to biohydrogenation of unsaturated fatty acid of rumen microbes. (Bauman et al., 1999). Linoleic acid (C18:2) were the precursors of CLA synthesis. The CLA was also synthesized in the mammary gland of lactating ruminants, using oleic acid (C18:1) as a precursor and activity of delta 9-desaturase (Griinari and Bauman, 1999). Linoleic acid is high in soybean oil (SO) (54.4%) and tuna oil (TO) (20.3%) while oleic acid is high in pork oil (PO) (43.5%) and groundnut oil (GO) (40.7%). Therefore, the objective of this experiment was to compare the increasing of CLA and fatty acid composition in milk fat form cows fed dietary oils obtained from either animal or plant sources.

**Materials and methods** Five mid-lactation multiparous crossbred Holstein cows (452±22 kg BW; 93±6 DIM) were used in a 5x5 Latin square design with five 24-d periods; 14d for adaptation, 7 d for data collection and 3 d for resting period. Cows were housed individually and milked twice daily at 0300 and 1500 h. Diets were supplied as a total mixed ration (TMR). Cows were offered control diet (CO) and oil diets containing 3.7%DM supplemental animal oil (TO and PO) or plant oil (GO and SO) in an equal amount at 0600, 1200 and 1700 h. Milk samples were obtained at each milking period during d 15 to d 20. Milk fat extraction was performed according to the procedure of Hara and Radin (1978). Fatty acid methyl esters were separated and quantified using gas chromatography. Data were analysis of variance using GLM procedures.

**Table 1** Fatty acid composition of experimental diets

| Fatty acid | | animal oil | | plant oil | |
|---|---|---|---|---|---|
| g/100g | CO | TO | PO | GO | SO |
| C14:0 | 0.01 | 0.41 | 0.32 | 0.23 | 0.82 |
| C16:0 | 19.33 | 20.52 | 28.61 | 17.91 | 19.23 |
| C18:0 | 11.50 | 5.71 | 7.30 | 4.81 | 0.08 |
| C18:1n9t | 23.1 | 19.92 | 3.72 | 24.33 | 17.67 |
| C18:1n9c | 0.02 | 0.03 | 19.33 | 26.05 | 0.00 |
| C18:2n6t | 24.72 | 20.87 | 0.00 | 0.01 | 0.00 |
| C18:2n6c | 15.11 | 19.35 | 28.18 | 17.17 | 53.29 |
| C18:3n6 | 0.09 | 0.12 | 0.39 | 0.56 | 1.21 |

**Results** The fatty acids concentration of each dietary treatment is shown in Table 1. The proportion of C8:0, C10:0, C14:0, C16:0, C18:0, C18:1n9t, C18:1n9c, 18:2n6t, 18:2n6c and C18:3n6 in milk fat of all cows were not affected by oil diets (Table 2). Average concentration of total CLA in milk fat was affected by oil diets, but the CLAc9t11 concentration was different by oil diets ($p<0.05$). Addition of plant oil (GO and SO) in diet resulted in an increase CLAc9t11 concentration in milk fat while animal oil (PO) did not affected CLAc9t11 concentration except TO

**Table 2** Milk fatty acid, and CLA component in milk fat form cows fed control diets and diets containing (3.7% of DM) animal or plant oils

| Fatty acid | | Animal oil | | Plant oil | | |
|---|---|---|---|---|---|---|
| g/100g | CO | TO | PO | GO | SO | SEM |
| C8:0 | 0.6 | 0.5 | 0.6 | 0.5 | 0.8 | 0.08 |
| C10:0 | 1.5 | 1.3 | 1.6 | 1.2 | 1.3 | 0.23 |
| C14:0 | 8.6 | 7.1 | 7.7 | 6.6 | 7.7 | 0.8 |
| C16:0 | 36.4 | 37.3 | 38.2 | 29.4 | 30.7 | 2.9 |
| C18:0 | 14.0 | 12.5 | 18.4 | 18.5 | 16.7 | 2.8 |
| C18:1n9t | 0.3 | 0.3 | 0.2 | 0.8 | 0.3 | 0.1 |
| C18:1n9c | 25.4 | 27.4 | 23.2 | 31.2 | 30.6 | 0.5 |
| C18:2n6t | 0.1 | 0.1 | 0.2 | 0.1 | 0.2 | <0.1 |
| C18:3n6 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 |
| 18:2 CLA | | | | | | |
| c9t11 | $1.3^{ab}$ | $2.2^{a}$ | $0.6^{b}$ | $2.4^{a}$ | $2.66^{a}$ | 0.44 |
| t10c12 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |
| total CLA | $1.8^{ab}$ | $3.3^{a}$ | $1.2^{b}$ | $2.5^{ab}$ | $3.1^{a}$ | 0.5 |

CO = control, PO = pork oil, TO = Tuna oil,
GO = groundnut oil, SO = soy bean oil,
$^{ab}$ = differ among oil diets ($P<0.05$)

**Conclusions** Fatty acid from animal and plant oil sources did not affect total-CLA and other fatty acids in milk fat. However, addition of TO, GO and SO in diet greatly increased concentration of CLAc9t11.

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**021**

# Changes in rumen microbial fermentation during acidosis are due to a combined effects of fermentation substrate and pH

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**Introduction** The supply of high-concentrate diets results in the reduction in ruminal pH and the development of acidosis. Because the reduction of pH occurs at the same time as the diet is changed, the effects are confounded. For example, high-concentrate diets ferment towards propionate, and low rumen pH also results in greater propionate production. Which factor is responsible for the increase in rumen propionate? The objective of this study was to determine the effects of rumen pH and the type of diet (D) on rumen microbial fermentation, with the aim of developing simple mathematical model to describe these effects.

**Material and Methods** Eight 1320-ml dual flow continuous culture fermenters were used in 4 periods to study the effects of rumen pH (4.9, 5.2, 5.5, 5.8, 6.1, 6.4, 6.7 and 7.0) and diet (FOR=60:40 forage:concentrate ratio; CON=10:90 forage:concentrate ratio) on microbial fermentation. Treatments were arranged in a 8 x 2 factorial. All other fermentation factors, including solid (5%/h) and liquid (10%/h) dilution rates and temperature (39ºC), were maintained constant. When fermenters were fed the FOR diet, they were inoculated with a composited rumen fluid from 2 dairy cattle fed the same diet. When fermenters were fed the CON diet, they were inoculated with a composited rumen fluid from 2 beef heifers fed a similar diet. A total of 100 g of DM were fed continuously throughout the day. Each experimental period consisted of 6 d for adaptation and 3 d for sampling. Samples were taken to determine ammonia N and VFA concentrations, digestion of OM, NDF, and CP, and flows of microbial and dietary N. Data were analyzed with a mixed-effects regression model with random intercepts and random slopes of pH. The pH was centred at 5.95 (for a value=0), and diets were assigned values of 1 (for FOR) or 2 (for CON). Polynomial equations were derived to determine the effect of diet and linear, quadratic, and cubic effects of pH, and their interactions, as fixed effects. Period was considered random effect. The final model was fitted so that only coefficients with $P$ values < 0.05 remained. Partial correlations of pH and diet, and their partial contribution to the variation explained by the model were calculated.

**Results** Polynomial equations are presented in Table 1. The pH was the major determinant on the true OM (TOMD; 85% of the variation) and NDF digestion (100% of the variation). In contrast, changes in total VFA concentration were explained by the combined effects of pH and diet (contributing 56 and 44%, respectively). Acetate concentration was mostly explained by changes in pH (98% of the variation), probably due to the decrease in fibre digestion with low pH (most fibrolytic bacteria are acetate producers). In contrast, propionate concentration was the result of the combined effects of pH and diet (55 and 45% of the variation, respectively). The variation in the acetate to propionate ratio was mainly explained by changes in pH (contributing 74%). Changes in N metabolism were explained mostly by the effect of diet (77% for NH3-N concentration; 73% for dietary N flow; 61% for microbial N flow; and 65% for protein degradation). Protein degradation in CON was lower than FOR in spite of diets being formulated with similar protein degradability, suggesting that current values of CP degradation (generally developed for dairy cattle) may not be correct if applied to high concentrate diets. The lack of effect of diet on TOMD was unexpected, and suggests that under the conditions of CON, degradation of feed was limited. The higher total VFA concentration in CON cannot be justified by similar TOMD or absorption (no absorption occurs *in vitro*), and can only be explained by a reduction in the utilization of carbons for microbial growth. In fact, microbial N flow was 26% lower and efficiency of microbial protein synthesis (EMPS, g microbial N/kg TOMD) was 10.9 units lower in CON than in FOR. The limited growth of bacteria may explain the lower than expected TOMD and the accumulation of VFA.

**Table 1** Polynomial equation of the effects of pH and type of diet on rumen fermentation

| | a | pH | $pH^2$ | $pH^3$ | D | DxpH | $DxpH^2$ | $DxpH^3$ | $R^2$ |
|---|---|---|---|---|---|---|---|---|---|
| TOMD, % | +50.2 | +14.9 | -8.71 | -9.68 | … | -13.7 | +7.02 | +10.7 | 0.79 |
| NDF, % | +24.2 | +19.1 | -5.70 | -10.7 | … | … | … | … | 0.60 |
| VFA, mM | +80.8 | +24.3 | -7.89 | … | +20.4 | … | … | … | 0.81 |
| Acetate, mM | +65.5 | +23.7 | … | … | -4.7 | … | … | … | 0.84 |
| Propionate, mM | +9.80 | -6.34 | -2.10 | +4.54 | +3.90 | … | … | … | 0.76 |
| A.P ratio | +4.61 | +1.93 | -0.49 | … | -1.66 | … | … | … | 0.89 |
| NH3N, mg N/dL | +16.5 | +6.90 | +1.98 | -2.14 | -7.47 | -3.34 | … | … | 0.96 |
| Diet-N flow, g/d | +0.90 | -0.58 | … | +0.29 | +0.54 | … | … | … | 0.45 |
| Bacterial N flow, g/d | +2.06 | +0.41 | -0.24 | -0.23 | -0.55 | … | +0.40 | … | 0.77 |
| CP degradation, % | +77.4 | +24.5 | -7.30 | -12.4 | -27.6 | … | +13.5 | … | 0.86 |
| EMPS | +46.3 | +2.34 | +2.68 | -9.68 | -10.9 | … | … | … | 0.71 |

a=Constant; pH, $pH^2$ and $pH^3$ = linear, quadratic and cubic effects of pH. D=diet effect.

**Conclusions** Changes in microbial fermentation due to high concentrate feeding are the result of the combined effects of the diet and changes in pH in different proportions depending on the item measured. These effects should be incorporated into models of rumen fermentation and may help to better understand rumen microbial metabolism. The degradation of OM in high concentrate diets appears to be limited by the growth of microbes. This hypothesis and its implication for the performance of high concentrate fed beef deserve further research.

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**022**

# Effect of incremental dietary fish oil inclusion on bacterial diversity in the rumen

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**Introduction** *In vitro* experiments have revealed that members of the *Butyrivibrio* group can convert linoleic (C18:2 *n*-6) and linolenic (C18:3 *n*-3) acid to VA (C18:1 *trans*-11), with some being able to further convert VA to stearate (C18:0). Advances in molecular microbial technology mean that we are now able to quantify these bacteria using quantitative PCR (qPCR) as well as look at total eubacterial and *Butyrivibrio*–specific population changes using Denaturing Gradient Gel Electrophoresis (DGGE). The aims of this study were to assess the involvement of *Butyrivibrio* spp. in the biohydrogenation pathways *in vivo* as well as investigate whether other bacterial species may be involved.

**Materials and methods** Six steers received a diet containing incremental fish oil concentrations (0, 1 and 3% of dry matter intake) in a 3 period Latin square design, as described by Kim *et al.* (2007). Fatty acid flow to the duodenum was assessed as described by Kim *et al.* (2007). Rumen digesta samples were collected, centrifuged (300 *g*, 10 min followed by centrifugation of the supernatant at 10,000 *g*, 25 min), before the resultant pellet was freeze-dried and ground. A general eubacterial as well as a *Butyrivibrio*-specific PCR-DGGE (Denaturation Gradient Gel Electrophoresis) was performed on the V6-V8 hypervariable region of 16S rRNA. DGGE gels were analysed using the software package Fingerprinting (Bio-Rad Laboratories), which was used to generate Unweighted Pair Group Method with Arithmetic mean (UPGMA) dendograms. qPCR targeting *Butyrivibrio* spp. producing stearate was also employed on digesta (Paillard *et al*. 2007). The effect of diet on stearate-producing *Butyrivibrio* 16S rRNA copies was assessed by analysis of variance (ANOVA). The relationship between stearate flow and *Butyrivibrio* 16S rRNA copies was examined by regression analysis (GenStat 9.1).

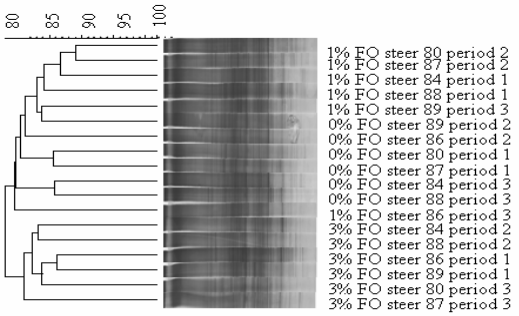


**Figure 1** DGGE-derived UPGMA dendogram showing the effects of fish oil on the total eubacterial population. FO-Fish oil. Scale - % similarity

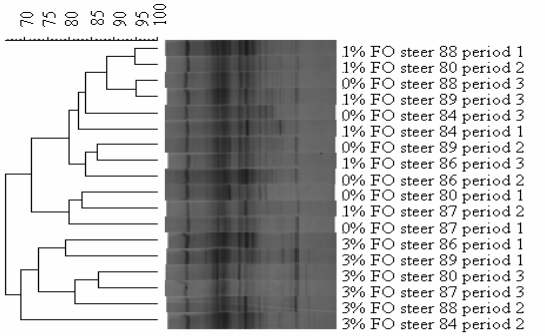


**Figure 2** DGGE-derived UPGMA dendogram showing the effects of fish oil on the *Butyrivibrio* population. FO - Fish oil. Scale - % similarity

**Results** Inclusion of fish oil was associated with a linear increase in VA (16.9 to 44.3 g/d) and a linear decrease in stearate (162.9 to 55.8 g/d) flow to the duodenum (Kim *et al*., 2007). The total eubacterial dendogram (Figure 1), as well as the *Butyrivibrio* dendogram (Figure 2), gave some indication that inclusion of fish oil caused some separate clustering of the bacterial community at the 3% concentration. However, ANOVA provided no evidence (P=0.260) to support an effect of fish oil on stearate-producing *Butyrivibrio* 16S rRNA copy number (31.8, 32.4 and 21.3 pg target DNA/mg sample dry weight respectively). A bootstrapped estimate of the correlation between stearate flow and stearate-producing *Butyrivibrio* qPCR data failed to provide evidence of association between the two variables (r=0.445 with 95% CI -0.052 to 0.773).

**Conclusions** Fish oil at the 3% intake concentration did cause some changes in the total and *Butyrivibrio*-specific bacterial community. However, whether we can attribute any of the decreases in stearate flow within this study to the stearate-producing *Butyrivibrio* is unclear, indeed a previous study revealed that the as yet uncultured bacteria may be playing a role (Huws *et al*., 2006). It should also be noted that the qPCR data is based on DNA which tells us little about the physiological status of the bacteria. Experiments are underway to clarify further the major bacteria involved in biohydrogenation, using both DNA and RNA as marker, with a view to developing strategies for manipulating this process leading to the beneficial enhancement in the nutritional value of ruminant products.

**Acknowledgements** This work was supported by the Biotechnology and Biological Sciences Research Council (BBSRC) and the Scottish Executive Environment and Rural Affairs Department (SEERAD).

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**023**

# Comparison of starch degradation in cereal grains by incubation *in vitro* with rumen fluid and boiling with α-amylase solution

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**Introduction** Cereal grains are the most common sources of readily available energy for dairy cows. Several *in vitro* and *in situ* studies have measured starch degradability of cereal grains. However, most of these studies compared few samples and grain species and used only one method. Compared with protein, much less information is available on this topic. Such information would allow a more efficient use of energy and protein in dairy cow diets. The objective of this study was to determine starch degradation of 32 samples of 7 cereal grains using *in vitro* incubation with rumen fluid and a new method based on a boiling extraction with an enzymatic (thermo stable α-amylase) solution.

**Materials and methods** Thirty two samples (2 kg each) of 7 cereal grains (2 barleys, 2 oats, 10 maize, 1 millet, 2 ryes, 13 triticale and 2 wheat) were used in this study. Grains were ground to pass 1 mm screen and analysed for DM and CP according to AOAC (1990) and NDF according to Van Soest *et al.* (1991). Samples ground to pass 0.5 mm screen were used for starch determination (Solomonson *et al*., 1984), *in vitro* incubations with rumen fluid and incubation with α-amylase. Rumen fluid was sampled 2 hours after the morning feeding from three non lactating fistulated cows. The daily ration of the donor cows consisted of maize silage, meadow hay and concentrate making 50:7:43 on DM basis. The rumen fluid was pooled and mixed 1:1 with a buffer solution. Thirty ml of the diluted rumen fluid was incubated with 500 mg of the sample. Incubations were conducted at 39ºC for 6 h in triplicate and were repeated four times. After incubation, 1 ml of 6N HCl was added to stop microbial activity and the tubes were centrifuged at 4000g for 15 min. The centrifugation was repeated twice and the residue was washed with hot distilled water. For the enzymatic method, 500 mg of sample were boiled for 30 minutes with 50 ml thermo stable α-amylase solution, in triplicate and were repeated twice. After boiling, the content was centrifuged as described above. Correlation between incubation *in vitro* with rumen fluid and after boiling with α-amylase solution was established.

**Results** Chemical composition of cereal grains (Table1) is in good agreement with current tabulated values. Differences in the *in vitro* degradation between cereal grains were found. The higher values were obtained for oat (68.8% starch, n=2) and the lower values were found for maize grains (55.8% starch, n=10). Average values of starch degradation (n=32) estimated after boiling with thermo stable α-amylase solution were slightly higher than values estimated by incubation *in vitro* with rumen fluid (61.83 and 60.20%, respectively) and the ranking of cereal grains according to starch degradation was similar with both methods. Correlation coefficient between degradation of starch estimated by boiling with thermo stable α-amylase solution or by incubation *in vitro* with rumen fluid was significant ($r=0.797$; $P<0.001$; $n=32$). The relationship was described by a linear regression: $y=1.0049\ x + 0.9553$, ($r^2=0.623$; rsd=3.561; $P<0.001$) where y is the starch degradation by incubation *in vitro* with rumen fluid and x is the starch degradation by boiling with thermo stable α-amylase solution.

**Table 1** Chemical composition of cereal grains

| Grains | DM (%) | CP (%DM) | NDF (%DM) | Starch (%DM) |
|---|---|---|---|---|
| Barley | 86.8 | 11.9 | 21.1 | 61.8 |
| Maize | 86.2 | 8.6 | 11.6 | 76.2 |
| Millet | 88.1 | 12.6 | 16.7 | 74.0 |
| Oat | 87.2 | 12.1 | 31.5 | 43.1 |
| Rye | 86.6 | 11.7 | 13,5 | 68.7 |
| Triticale | 87.2 | 12,0 | 15.3 | 55.6 |
| Wheat | 86.0 | 12.2 | 14.7 | 73.2 |

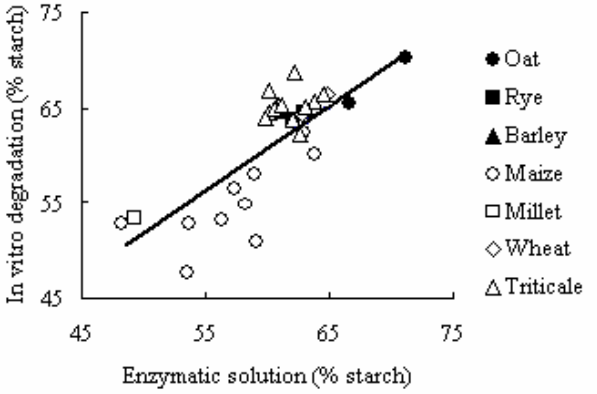


**Figure 1** Relationship between degradation of starch estimated by boiling with thermo stable α-amylase solution or by incubaion *in vitro* with rumen fluid.

**Conclusions** These results show that boiling with thermo stable α-amylase solution for 30 min can adequately estimate starch degradation of cereal grains by incubation *in vitro* with rumen fluid for 6 h. Further studies are needed to compare these methods with the *in situ* method.

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# Relations between ruminal and intestinal *in sacco* starch digestion in ruminants

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**Introduction** The proportion of the starch that escapes rumen degradation varies from 5 to 65% of the starch intake. It depends on the feeding level and the nature of the cereal, and for a same cereal, on its endosperm hardness, related to genotype and maturity, and on the technological treatments, including particle size (Huntington et al., 2006). The intestinal digestion of starch escaping rumen degradation essentially takes place in the small intestine and varies between 15 to 85% of the starch that arrives into the duodenum. As in the rumen, this variability would essentially be due to the intrinsic features of the maize that determines the accessibility of the starch to the endogenous enzymes, in particular the endosperm hardness and the particle size. The aim of this work was to evaluate the rumen starch degradation of maize and its residue digestion in the small intestine as function of particle size and endosperm hardness.

**Materials and methods** Six maize grain (*Zea mays*) genotypes characterised by different endosperm hardness (proportion (%) of vitreous on total endosperm, determined by NIRS, Limagrain Genetics, France) were ground (3-mm screen; SP, small particles, mean particle size (MPS): 526 µm) and cracked with a roller mill using 2 gap width settings (MP, medium particles, MPS: 1360 µm; LP, large particles, MPS: 2380 µm). Three dry Holstein cows, fitted with rumen, proximal duodenum and terminal ileum cannulas, were fed maize silage *ad libitum*, twice daily at 0700 and 1630 h. Experimental maize grain (3 g) was put into nylon bags (p.s. 53 µm; i.d.: 5x10 cm) and incubated for 3, 6, 9, 15, 24 and 48 hours in the rumen. Residues from 15 hours incubation were put in small nylon bag (i.d.: 4x3 cm, p.s. 53 µm, 1,2g/bag) containing an iron ball (6 mm diameter), and introduced into the duodenum (10 min intervals, randomised order) following a 1.5 h incubation in a pepsin-HCl solution. A magnet was used to take out the bags through ileal cannula. Experimental feeds, ruminal and intestinal residues were analysed for starch (Faisant et al., 1995). Starch ruminal degradability was calculated by the step-by-step method with a fixed passage rate of 0.06 $h^{-1}$. Data were analysed with the mixed procedure of SAS, with genotype, particle size and their interactions as fixed effects, and animal as random effect. For intestinal digestibility, the time between duodenal and ileal passage was introduced as covariable in the model.

**Results** The ruminal starch degradability and intestinal digestibility varied among genotypes (P<0.001) and decreased when particle size increased (P<0.001)(Table 1). For a same particle size, starch ruminal degradability tended to decrease (P=0.17) and intestinal digestibility decreased (P<0.002) with endosperm hardness. The estimation of the proportion of starch intake digested in small intestine = ((1-rumen degradability)*small intestine digestibility) showed an optimum level of rumen starch degradation which allows to maximise the fraction of starch intake digested in the small intestine (Figure 1).

**Table 1** *In sacco* rumen degradability (%) and intestinal digestibility (% of duodenal incubation) of starch for maize grains differing in endosperm hardness (EH) and particle size.

| | Particle size | | |
|---|---|---|---|
| | LP | MP | SP |
| EH | Rumen degradability | | |
| 35 | 35,1 $^{Bb}$ | 39,9 $^{BCb}$ | 62,3 $^{ABa}$ |
| 41 | 42,5 $^{Ac}$ | 49,6 $^{Ab}$ | 63,2 $^{Aa}$ |
| 47 | 32,8 $^{Bb}$ | 48,1 $^{Aa}$ | 45,7 $^{Ca}$ |
| 55 | 37,3 $^{ABc}$ | 48,8 $^{Ab}$ | 65,8 $^{Aa}$ |
| 64 | 23,7 $^{Cc}$ | 35,9 $^{Cb}$ | 41,9 $^{Ca}$ |
| 68 | 34,3 $^{Bc}$ | 43,9 $^{ABb}$ | 57,3 $^{Ba}$ |
| | Small intestine digestibility | | |
| 35 | 61,4 $^{Ac}$ | 76,5 $^{Ab}$ | 84,3 $^{Aa}$ |
| 41 | 63,2 $^{Ac}$ | 71,2 $^{BCb}$ | 85,1 $^{Aa}$ |
| 47 | 51,5 $^{Cc}$ | 73,5 $^{ABb}$ | 79,3 $^{Ba}$ |
| 55 | 56,4 $^{Bc}$ | 69,8 $^{Cb}$ | 84,3 $^{Aa}$ |
| 64 | 39,2 $^{Dc}$ | 60,6 $^{Db}$ | 73,7 $^{Ca}$ |
| 68 | 49,3 $^{Cc}$ | 69,4 $^{Cb}$ | 79,5 $^{Ba}$ |

Means within a row (small letter) or within a column (capital letter) with different superscript differ (P<0.05); LP, large particles; MP, medium particles; SP, small particles.

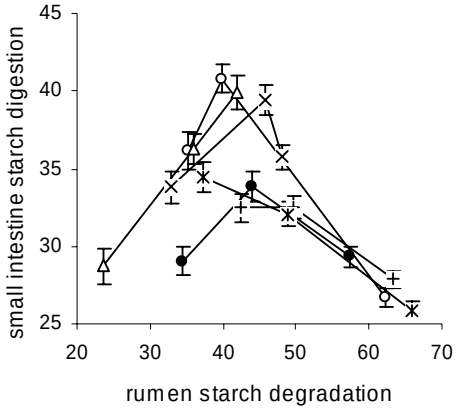


**Figure 1** Relation between estimated ruminal and small intestinal starch digestion (% intake). Endosperm hardness (%) : 35 (○); 41 (+); 47 (x); 55 (*); 64 (Δ); 68 (●).

**Conclusions** The particle size and endosperm hardness of maize grain are efficient factors to manipulate the amount of starch escaping rumen degradation but may constitute limiting factors in the amount of starch digested in the small intestine.

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**025**

## Red clover polyphenol oxidase reduces ruminal lipolysis in *in vitro* batch culture

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**Introduction** It has been shown that the rate of lipolysis and proteolysis differs significantly between red clover genotypes with different levels of polyphenol oxidase (PPO) activity (Lee et al. 2004). Sullivan and Hatfield, (2006) reported the development of genetically modified isolines of red clover with the PPO1 gene silenced. This material was used to examine the role of the red clover PPO enzyme on lipolysis and ultimately C18 polyunsaturated fatty acid biohydrogenation in batch culture. If the role of PPO in reducing ruminal lipolysis of plant lipids is proven it would influence breeding strategies for forages which exhibit this trait in an attempt to increase the levels of beneficial PUFA and decrease detrimental trans and saturated fatty acids in animal products.

**Materials and methods** Anaerobic incubation medium was prepared as described by Goering and Van Soest (1970) and 20ml dispensed under $CO_2$ into 24 flasks maintained at 39$^o$C. Fresh red clover from silenced PPO1 gene plants (S) and isogenic active PPO1 gene plants (A) were cut 3 cm above soil level. The tissue was crushed and cut into 5 mm strips, with a sample retained at -20$^o$C to measure PPO activity. Four grams of fresh material was loaded into each incubation flask. Three bottles were allocated to each time point (0, 1, 6 and 24 h) for each treatment (S and A). The flasks were then inoculated with 10 ml of strained rumen liquor (from two rumen fistulated cows and strained through a double layer of muslin). The flasks were sealed and incubated at 39$^o$C in the dark with continuous $CO_2$ purging. At each time point the appropriate incubation flasks were removed and 40 ml of isopropanol : chloroform (1:1 v/v) along with 1 ml of internal standard (2.5 mg C23:0 / ml chloroform) added and the lipid extracted and fractionated by TLC as described by Lee et al. (2004). Lipolysis was calculated by expressing the decrease in the proportion of membrane lipid between the initial time point $T_0$ and incubation time point $T_x$, and then analysed using a repeated measures analysis of variance (Genstat 8.1, Lawes, Agricultural Trust, 2005).

**Results** PPO activity for the PPO1-silenced red clover (S) and the active PPO1 red clover (A) were 0 and 11.4 nkat/mg protein. Fig. 1. shows the extent of lipolysis of S and A. At all time points lipolysis in the S treatments were higher than the A treatments with 6 and 24 h being significantly higher ($P<0.001$) than S.

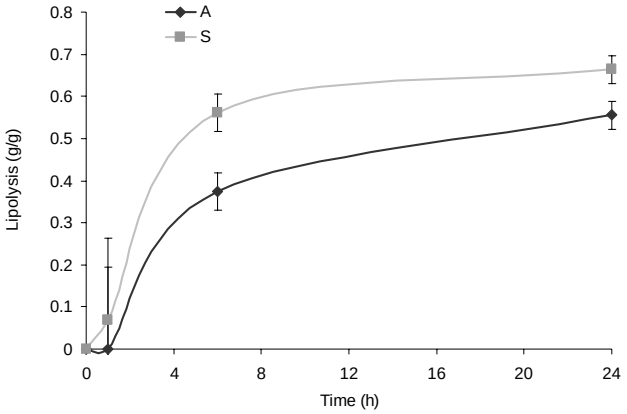


**Figure 1** Lipolysis of the genetically modified red clover (S) and the non-genetically modified red clover (A) incubated in batch culture with rumen liquor at 39$^o$C for 24 h.

**Conclusions** The PPO1-silenced red clover had significantly higher lipolytic activity in batch culture than the red clover with the active PPO1 gene. This result, obtained with isogenic lines, provides strong evidence for a role of PPO in reducing the extent of lipolysis in the presence of rumen micro-organisms. Mechanistically this may be due to a binding of quinones to the lipid or/and the formation of protected protein/lipid complexes

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**026**

# Accumulation of biohydrogenation intermediates during *in vitro* ruminal fermentation of camelina oil-based rations

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**Introduction** The myriad putative health benefits of conjugated linoleic acid (CLA) and in particular the cis-9, trans-11 isomer, have stimulated interest in increasing its concentration in food. Ruminant fat is the main dietary source of CLA for humans and CLA is produced in the rumen by incomplete biohydrogenation of dietary linoleic acid (LA). It is now accepted that most CLA is synthesised post-ruminally by desaturation of vaccenic acid (VA) produced during ruminal biohydrogenation of (LA) and linolenic acid (LNA) (Griinari *et al*., 2000). Enhancement of VA synthesis in the rumen is therefore an important element of strategies to increase CLA concentration in tissue. The objective of this experiment was to determine the effect of controlling the rate of release of oil from camelina seeds, a novel source of both LA and LNA, on the accumulation of intermediates during ruminal biohydrogenation.

**Materials and methods** Five rations formulated to contain 60g lipid/kg of dry matter were examined. The main lipid sources were; 1) megalac (MG), (control), 2) camelina oil (CO), 3) camelina seed (CS); (treated with 100g NaOH/kg seed), 4) camelina amide (CA); (CO reacted with 0.73g ethanolamine/g oil) and 5) Ground CS (CSG), (ground in a Vita Mix 3600, set at speed 4 for 20 seconds per 100g, to simulate mastication). Samples were incubated (in duplicate) in rumen fluid from six cannulated ewes for 0,3,6,10,16 and 24 hours at 39$^{o}$C in three separate runs, according to Chow et al. (2004). Lipids were extracted using chloroform methanol (C/M, 2/1, v/v), dried under nitrogen, methylated and fatty acid methyl esters (FAME) were analysed by gas chromatography. Data were subjected to ANOVA and all pair-wise comparisons were carried out using Tukey's t-test.

**Results** Fatty acid proportions (in total lipid) after 3, 6 and 10 hours incubation are summarised in Table 1. CA caused the greatest increase in CLA trans-10, cis-12, CLA cis-9, trans-11 and VA concentrations up to six hours and VA concentrations over all time-points shown. VA concentrations were lowest for CS at all time-points reported. On average, accumulation of CLA and VA was similar (P>0.05) for the oil and whole seeds and for whole and ground seeds.

**Table 1** Effect of ration and incubation time on fatty acid composition (g/kg)

| Treatment | MG | CO | CS | CA | CSG | SED | Sig. |
|---|---|---|---|---|---|---|---|
| *3 Hours* | | | | | | | |
| C18:2n6 | 130.4$^{c}$ | 175.5$^{b}$ | 170.6$^{b}$ | 220.3$^{a}$ | 169.1$^{b}$ | 11.2 | *** |
| C18:3n3 | 78.8$^{b}$ | 286.8$^{a}$ | 331.9$^{a}$ | 132.3$^{b}$ | 297.6$^{a}$ | 43.3 | *** |
| CLA 10,12 | 0.70$^{b}$ | 0.10$^{b}$ | 0.10$^{b}$ | 2.20$^{a}$ | 0.70$^{b}$ | 0.30 | *** |
| CLA 9,11 | 0.80$^{b}$ | 1.00$^{b}$ | 1.10$^{b}$ | 7.80$^{b}$ | 0.80$^{b}$ | 1.60 | *** |
| VA | 1.28$^{b}$ | 1.14$^{b}$ | 1.13$^{b}$ | 4.01$^{a}$ | 1.84$^{ab}$ | 0.89 | * |
| *6 Hours* | | | | | | | |
| C18:2n6 | 112.9$^{c}$ | 168.8$^{b}$ | 164.0$^{b}$ | 202.0$^{a}$ | 163.4$^{b}$ | 10.8 | *** |
| C18:3n3 | 1.14$^{d}$ | 26.85$^{b}$ | 31.80$^{a}$ | 10.70$^{c}$ | 27.82$^{b}$ | 1.19 | *** |
| CLA 10,12 | 0.40$^{b}$ | 0.20$^{b}$ | 0.50$^{b}$ | 2.10$^{a}$ | 0.60$^{b}$ | 0.20 | *** |
| CLA 9, 11 | 0.20$^{b}$ | 1.20$^{b}$ | 2.40$^{b}$ | 7.20$^{a}$ | 3.00$^{ab}$ | 1.50 | *** |
| VA | 22.2$^{b}$ | 24.4$^{ab}$ | 18.0$^{b}$ | 66.4$^{a}$ | 24.5$^{ab}$ | 14.9 | * |
| *10 Hours* | | | | | | | |
| C18:2n6 | 101.7$^{b}$ | 138.1$^{ab}$ | 148.1$^{a}$ | 158.9$^{a}$ | 135.2$^{ab}$ | 12.8 | ** |
| C18:3n3 | 10.5$^{d}$ | 206.6$^{b}$ | 275.1$^{a}$ | 72.2$^{c}$ | 197.0$^{b}$ | 16.8 | *** |
| CLA 10,12 | 0.60$^{b}$ | 0.30$^{b}$ | 5.10$^{b}$ | 3.70$^{a}$ | 0.70$^{b}$ | 0.40 | *** |
| CLA 9, 11 | 0.10$^{b}$ | 2.90$^{ab}$ | 9.40$^{ab}$ | 3.40$^{ab}$ | 6.80$^{a}$ | 1.50 | ** |
| VA | 36.0$^{b}$ | 48.8$^{b}$ | 35.8$^{b}$ | 98.9$^{a}$ | 56.9$^{ab}$ | 16.3 | ** |

CLA 9,11 = CLA cis-9, trans-11; CLA 10,12 = CLA cis-10, trans-12.
***<0.001; **<0.01; *<0.05. Within a row, means not sharing a common superscript are significantly different.

**Conclusion** Altering the rate of release of camelina oil to rumen fluid *in vitro* influenced the pattern of dietary fatty acid metabolism. Chemical protection of oil with ethanolamine was most effective in increasing the accumulation of VA suggesting that it was not effective in reducing the rate of biohydrogenation. Accumulation of VA for CS tended to be lower compared to CO indicating that oilseeds offered more protection than oils to ruminal biohydrogenation. The greater accumulation of VA for CSG compared to CS suggests that processing the seed prior to incubation increased the rate of biohydrogenation compared to the unprocessed seed.

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**027**

# Determination of protein digestibility of animal protein feeds under condition of *in vitro* and *in vivo*

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**Introduction** Artemia or brine shrimp is a crustacean animal that lives in conditions of high salinity in the tropical and sub-tropical regions of the world. There is great potential for the culture and production of artemia in Iran. Artemia biomass is a good source of protein that can be used in poultry diets (Zarei *et al.*, 2006). The objective of this study was to determine the protein digestibility of different sources of artemia *in vitro* and *in vivo* in comparison with fish meal.

**Materials and methods** Artemia biomass was harvested from three regions of Iran: Urmia lake (ULA); ponds around the edge of Urmia lake (EPA) and from Ghom salt lake (GSLA). Samples were dried at 50-60°C, milled, and chemical composition determined. *In vitro* protein digestibility was evaluated by digestion with pepsin (AOAC, 1996). *In vivo a*pparent and true digestibility of the three sources of artemia meal and of fish meal were evaluated using 96 male chicks (Ross 308 broiler strain) with 3 replicates of chicks (8 chicks / replicate) per treatment. Digestibility was determined by ileal sampling with chromic oxide used as a digestion marker. Artemia or fish meal were included in diets as the sole source of protein and fed from 21 days of age. After 7 days adaptation, excreta were collected from 28 to 33 days of age. Birds were then sacrificed with $CO_2$ and samples collected from the terminal ileum. Apparent and true digestibilities of protein were calculated as described by Kadim *et al* (2002). Data were analyzed by Anova using SAS and means compared using Duncan's multiple range test.

**Results** The artemia samples contained 402, 391 and 423 g crude protein and 135, 86 and 207 g ether extract /kg DM for ULA, EPA and GSLA respectively. Values for fishmeal were 679 g crude protein and 207 g ether extract / kg DM. Both in vitro and *in vivo*, ULA artemia was less digestible than other samples of artemia ($P<0.05$; Table 1). There was no differences in digestibility between EPA and GSLA artemia which had digestibilities similar to fish meal.

**Table 1** *In vitro* and *in vivo* protein digestibilities of artemia samples (g/kg protein)

| Treatment | *In vivo* | | | | *In vitro* | |
|---|---|---|---|---|---|---|
| | Apparent digestibility | SD | True digestibility | SD | Pepsin digestibility | SD |
| ULA | 830[b] | 1.4 | 869[b] | 1.4 | 905[b] | 0.8 |
| EPA | 869[a] | 2.9 | 913[a] | 0.9 | 919[a] | 0.2 |
| GSLA | 858[a] | 2.0 | 902[a] | 2.0 | 927[a] | 0.5 |
| Fish meal | 880[a] | 1.0 | 921[a] | 1.9 | 921[a] | 0.2 |

Means in same row with different superscripts are significantly difference ($P< 0.05$)

**Conclusions** Artemia meal can be used as a feedstuff in poultry diets as it contains high concentrations of protein of similar protein digestibility to fishmeal. Compared with other animal proteins, artemia does not contain any feather, bone, hair or gastrointestinal tract components. In addition to produce artemia, there is no requirement for high pressure and high temperature treatment.

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# Sites of phytase and xylanase activities in the gastrointestinal tract of broiler chickens

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**Introduction** Because digesta vary in pH in different gastrointestinal segments of poultry, exogenous phytase or xylanase may exhibit differences in activity along the gastrointestinal tract. Previous reports indicated that the stomach is the major site of exogenous microbial phytase activity, with no further activity found in the small intestine of piglets. Information regarding exogenous phytase or xylanase activity in the gastrointestinal tract of poultry is largely unavailable. Because exogenous phytase or xylanase activity in the digesta is extremely low, normal phytase or xylanase activity measurements are prone to errors resulting from background interference contributed by the exogenous inorganic phosphate or xylose in the digesta (Walsh *et al.,* 1995). The aim of this study was to utilize electrophoresis activity stain to detect the activity of phytase or xylanase in different gastrointestinal segments of broiler chickens fed diets containing exogenous enzymes.

**Materials and methods** Ninety day-old broilers were selected and randomly allocated to diet (12.54 Mj ME, 180 g/kg CP, 43 g/kg ether extract) based corn-soyabean meal without enzyme, with phytase or xylanase supplementation. At 3 and 6 weeks of age, fifteen chickens from each treatment were slaughtered and the crop, gizzard, duodenum, jejunum and ileum contents were emptied and collected for electrophoresis and activity staining. The normal spectrophotometer methods for phytase (Bae *et al.,* 1999) and for xylanase (Bailey and Poutanen, 1989) were conducted on collected samples of all treatments. Proteins extracted from the various digesta samples were resolved using SDS-PAGE using 12.5% polyacrylamide in the separating gel and 4.75% in the stacking gel and native-PAGE using 7% polyacrylamide in the separating gel. After SDS-PAGE, the gel was stained with Coomassie blue R-250 and destained. After native-PAGE, the gel related to phytase activity stain was dipped into 5.1 mM sodium phytate and incubated at 37°C for 1 h with stop reagent added. The phospho-molybdate complex was reduced using iron sulfate and a blue complex was formed. The blue color reflected the phytase activity. Xylanase activity in native-PAGE gels was detected by overlaying the gel with 2% agar dissolved in 100 mM MES buffer, pH 6.5, containing 1.5% Remazol Brilliant Blue-Xylan. The color developed in the gel as a single band reflected the xylanase activity. Data were analyzed as a completely randomized design using the general linear model procedure of SAS (1996).

**Results** The normal spectrophotometer method failed to detect exogenous phytase activity in the diet or in the digesta of broiler chickens fed the phytase or xylanase diet. Exogenous phytase or xylanase protein was detected by SDS-PAGE in the phytase or xylanase diet as well as in the digesta of crop, proventriculus, gizzard, duodenum and jejunum of broiler chickens fed the phytase or xylanase diet. On the other hand, exogenous enzyme activity was detected by the activity stain native-PAGE assay in the phytase or xylanase diets and the digesta collected from the crop, gizzard and duodenum with the exception of jejunum and ileum. The result of the study suggests that the activity stain assays allow the detection of low levels of exogenous phytase or xylanase activity in the diet as well as in the digesta collected from the gastrointestinal tract of the broiler chicken (Table 1). Phytase activity in digesta from the crop was higher than that from proventriculus or that from gizzard ($P$<0.05).

Table 1. Phytase and xylanase activity (%, as proportion of the total activity measured) in different segments of gastrointestinal tract.

| | Phytase | Xylanase |
|---|---|---|
| Crop | 55[a] | 45[a] |
| Proventriculus | 35[b] | 40[b] |
| Gizzard | 10[c] | 15[c] |
| S.E.M | 1.9 | 1.7 |

No activity detected in the small intestine. Increased degradation of the phytase or xylanase protein resulted in no detectable phytase or xylanase protein or activity from SDS-PAGE and activity on stain gel. Moreover, differences in pH and the degree of endogenous protease action results in exogenous phytase or xylanase exhibiting varying activities in different segments of the gastrointestinal tract.

**Conclusions** It is concluded that crop and proventriculus in poultry were the major functional sites of exogenous phytase and xylanase. The SDS-PAGE and native-PAGE activity stain assays allow the detection of low levels of exogenous phytase or xylanase activity in the diet as well as in the digesta collected from the gastrointestinal tract of the broiler chicken.

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**029**

# Variability in semen quality from British sport horses

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**Introduction** Recent advances in artificial insemination (AI) and semen preparation have led to an increase in the availability of equine genetics globally. Generally, less than half the stallion population produce semen of suitable robustness to maintain acceptable fertility post-freezing, and research is needed to improve not only the numbers of stallions whose semen can be successfully frozen, but also to increase the number of collections that meet fertility standards. The number of progressively motile sperm present during ovulation determines successful conception in horses. AI practitioners recommend that 500 million progressively motile sperm should be introduced into the mare's uterus to ensure best chance of pregnancy (Matson and Pycock, 2006), and progressive motility is currently accepted as suitable for determining fertility in stallions. Established laboratory quality assessments are used to identify stallions with suitably robust semen for freezing and worldwide distribution, however fertility traits can be influenced by many factors, e.g. age, level of competitive performance or workload, nutrition, disease and stress (Tucker and Morris, 2006). In order to investigate the impact of such factors on semen quality, it is first important to determine the level of variation in semen quality between stallions that are currently used for frozen semen collections, which formed the main objective of the following study. This baseline can then be used to compare improvements in semen quality from various nutritional and management interventions, which form the basis of our ongoing research program.

**Materials and methods** Six stallions were randomly selected from collections made at Stallion AI Services between October 2005 and October 2006. The horses received the same housing, management and premium commercial feed, with intake adjusted for body weight (which was monitored twice weekly throughout the collection period). 'Flushing' collections were made prior to the main collecting period, to remove senescent sperm according to recommended practice. Semen was collected in an artificial vagina from sires covering a dummy mare (Matson and Pycock, 2006) at several time points during their confinement at the AI centre. Each batch was filtered to remove the gel fraction. Semen volume and sperm density were measured, and quality characteristics (motility, velocity and abnormalities) were determined by microscopy. Semen from each collection was packed into 0.5 ml straws and frozen, after which three samples per batch were randomly selected, and thawed. Semen was removed from the straws and assessed for morphological abnormalities and motility (scored on a 0-10 scale) immediately post-thaw and after 60 minutes on a heated slide (37ºC). Semen batches were graded as 'pass' or 'fail' for fertility, based on percentage of progressively motile sperm post-thaw. Each batch of semen collected was treated as an individual result for that sire, and data was analysed by one-way ANOVA using the GLM process of UNISTAT, with confidence limits set at 5%. .

**Results** Table 1 shows the results for the semen collections and sperm quality assessments. Semen attaining quality standards required for fertility (based on progressive motility) varied considerably between sires during the collection period, ranging from 40% to 92%. Significant differences were observed between stallions for all sperm parameters. Interestingly, fresh motility was not directly correlated to motility of semen post-thaw. Motility reduction during the 60 minutes post-thaw period also varied between stallions, with scores reducing by only 15% in sire 4 and up to 36% in sires 2 and 6. In practise, equine AI normally utilises semen immediately post-thaw, however the 60 minute evaluations provide a guide to potential robustness *in utero*.

**Table 1** Variation in fresh and frozen semen quality from six stallions

| Parameter | Sire 1 | Sire 2 | Sire 3 | Sire 4 | Sire 5 | Sire 6 | P value | SEM |
|---|---|---|---|---|---|---|---|---|
| Total collections (n) | 28 | 23 | 12 | 14 | 25 | 14 | - | - |
| Batches achieving 'Pass' (%) | 68 | 52 | 92 | 86 | 40 | 57 | - | - |
| Post thaw live sperm (%) | 47.9 $^{a}$ | 54.4 $^{b}$ | 53.0 $^{ab}$ | 57.2 $^{b}$ | 45.6 $^{a}$ | 45.9 $^{a}$ | <0.001 | 0.83 |
| Fresh motility (0-10*) | 6.42 $^{b}$ | 6.32 $^{b}$ | 6.18 $^{ab}$ | 5.88 $^{a}$ | 6.13 $^{ab}$ | 6.29 $^{ab}$ | 0.007 | 0.044 |
| Post-thaw mot. 0min (0-10*) | 3.65 $^{ab}$ | 3.58 $^{ab}$ | 3.73 $^{ab}$ | 3.92 $^{b}$ | 3.33 $^{a}$ | 3.66 $^{ab}$ | 0.002 | 0.038 |
| Post-thaw mot. 60min (0-10*) | 2.61 $^{ab}$ | 2.29 $^{a}$ | 3.05 $^{bc}$ | 3.42 $^{c}$ | 2.30 $^{ab}$ | 2.33 $^{ab}$ | <0.001 | 0.078 |
| Post-thaw velocity (0-5*) | 3.0 $^{bc}$ | 2.8 $^{ab}$ | 3.3 $^{cd}$ | 3.7 $^{d}$ | 2.6 $^{a}$ | 3.3 $^{cd}$ | <0.001 | 0.05 |
| Acceptable sperm (0-10*) | 3.64 $^{ab}$ | 4.44 $^{c}$ | 3.88 $^{abc}$ | 4.20 $^{bc}$ | 3.28 $^{a}$ | 4.05 $^{abc}$ | <0.001 | 0.08 |

$^{a, b}$ means not sharing a letter differ significantly (P<0.05) *based on an established scoring system

**Conclusions** The number batches of semen attaining suitable quality to be 'passed' for frozen AI use was shown to vary widely in the six individual horses assessed in this trial. Understanding variability in equine semen quality is crucial to ensure breeders can select sires by their fertility and, hence, improve conception rates. This finding forms the basis for future investigations regarding factors affecting semen quality as a means to improve the number of horses producing semen of suitable robustness for freezing, and also to reduce the number of semen collections that fail on quality parameters. Such advances will increase the genetics available to horse breeders worldwide, and will make the semen collection more cost effective for stallion owners.

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**030**

# Mare age as a selection consideration for sport horse broodmares; a breeder survey

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**Introduction** Selection considerations for mares for breeding are varied. Most breeders consider factors such as performance, pedigree, progeny and conformation very carefully. It has been suggested (Roberts, 1986) that fertility rates may be comparatively poor (under 50% per cycle) in the aged (over 15 years) broodmare. This study (via survey) examined) the factors considered by event horse breeders when selecting mares for breeding and their stated knowledge of reproduction and physiology.

**Materials and methods** A postal questionnaire was sent to breeders of event horses. Breeders were asked to grade the importance of 10 stated criteria when they selected mares for breeding. Grading was scaled between one, very unimportant to five very important. Additionally respondent were asked to classify their own knowledge of mare reproduction and physiology (average or less, good or greater). Chi squared analysis was performed on data relating to reproductive physiology knowledge and relative importance of mares age.

**Results** There were 57 respondents to the 120 postal questionnaires sent out, 47.5%. Mare temperament was seen as the most important consideration by breeders (table 1), with mare age as the least important consideration for selection. Further investigation into mare's age was conducted; breeders were asked to state their knowledge of mare reproduction and physiology. Of the respondents 74% (n=42) believed they had good or greater knowledge of mare reproduction and physiology, whilst only 26% (n=15) stated they had an average or lesser knowledge. Figure 1 represents the relative importance placed by breeders on mare age in selection.

**Table 1** Relative importance of selection factors for mares

| Breeding Consideration. | Score. |
|---|---|
| Temperament of the Mare | 4.74 |
| Mare Conformation | 4.40 |
| Mare Movement | 4.39 |
| Performance History | 4.35 |
| Mare Soundness | 4.21 |
| Mare Pedigree | 4.13 |
| Performance of Progeny | 4.12 |
| Mare Reproductive Conformation | 4.07 |
| Mare Breed/Type | 3.70 |
| Mares Age | 2.94 |

**Table 2** Cross tabulation of breeder knowledge and mare age

| | | Importance of mares age | | |
|---|---|---|---|---|
| | | V. importance or important | Neutral | Unimportant |
| Knowledge of mares reproductive physiology | Good or greater | 14 | 20 | 8 |
| | Average or less | 0 | 6 | 9 |

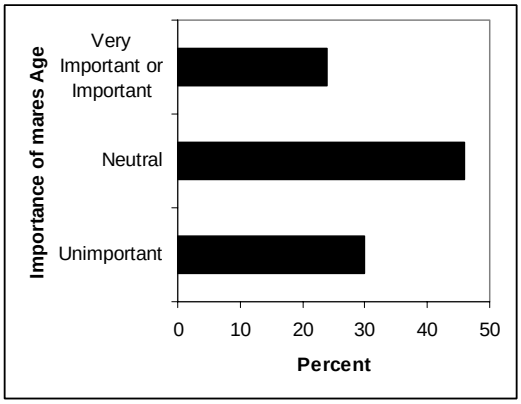


**Figure 1** Importance placed on mares age by breeders

Cross tabulation of stated reproductive knowledge and importance of mares age (Table 2) shows 25% (n=14) of breeders who stated they had good or greater knowledge thought that mare age was either very important or important, whilst 14% believed it was unimportant. The $\chi^2$ analysis returned a non significant value (0.128) for mare age, whilst a significant effect ($p<0.001$) was observed for breeder knowledge.

**Conclusions** The survey demonstrates that breeders are placing a high importance on a number of 'traditional' factors (temperament, conformation, movement, performance, soundness, pedigree, progeny and reproductive conformation) when considering selection of mares for breeding. It noted that mare's age is thought the least important consideration by a considerable margin. When this response is considered against breeders stated knowledge only a quarter of the population state they have good or greater than good knowledge believe mare age to be very important or important. However 14% of this category think age is unimportant, with in total 30% of the population believing this to be the case. It is also interesting to note that when breeders are asked to state the importance mare reproductive conformation they rate it at 4.07 whilst they rate age at 2.94. This may indicate that some breeders may not be aware of the established link between age and reproductive soundness.

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**031**

# Prevention of forelimb lameness and splint formation in young sport horses: A preliminary study and comparison of two pasture management systems

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**Introduction** Good conformation and inherent soundness are major prerequisites in the performance horse (Holmstrom & Drevemo, 1997). One of the most common conformational faults associated with the equine forelimb is splint formation. Particularly in the case of young horses (<5 years old), the formation of splints along the medial and lateral aspects of the cannon bone (3rd metacarpal) is often a cause of lameness. The splint bones are the smaller 2nd (inner or medial) and 4th (outer or lateral) metacarpal bones that rest along-side the cannon bone and the inner splint bone tends to be more frequently affected. Several conditions/activities such as inappropriate nutrition, hard ground, direct trauma as in the opposing hoof hitting the inside splint bone of the opposite leg and poor conformation (off-set knees) create an increased risk of splint formation. Pasture management is an important feature of horse production and growing horses spend much of the year at grass. While at pasture, overly excessive play and motor activity (slipping, falling and jumping) might also induce splint formation and lameness. Splints generally result from inflammation of the ligament that knits the splint and cannon bones together leading to inflammation of the periosteum (periostitis) and new bone formation at the damage site. In cases where substantial blunt trauma occurs, it may also involve the cannon bone and the occurrence of single or multiple swellings. Newly formed splints can cause degrees of lameness of the affected limb, but rest is usually sufficient to overcome the damage. The objective of this study was to compare two different grassland husbandry systems for rearing young sport horses with regard to the prevention of occasional lameness and splint formation in the yearling while at summer pasture.

**Materials and methods** Irish sport horse yearlings (n = 49) participated as subjects for this study at two Irish stud farms; (n = 27) on farm A and (n = 22) on farm B. The horses were castrated males, aged 11 $\pm$ 0.6 months at the start of the study and there was no evidence of either lameness or splints on any animal when they were initially turned out to summer grass. Farm A animals had been wintered in a single group on straw bedding in an enclosed 15m × 7m housing unit and had continual access to a 15m × 7m concrete yard with haylage and water *ad libitum* for the winter season (November to April). This group were subsequently released to a 17 hectare out-farm (all in one unit) for summer grazing (May to October). Farm B animals were wintered in two sub-groups (n = 10 and n = 12) on straw bedding in two adjoining winter housing units; each unit measured 7m × 7m and the horses also had access to water and similar quality haylage *ad libitum*. The horses on farm B were turned out into exercise sand paddocks from 9am to 3pm every day during the housing season. The animals on farm B were released together in one group to intensely managed grazing paddocks for the summer season (similar herbage to Farm A) and each paddock was approximately 1.2 hectares. The horses were rotated through 7 paddocks in total during this time to meet the demand for forage. Rotation ranged from four to approximately nine days per paddock. All horses on each farm were routinely inspected every day during the summer grazing season and all incidences (and duration) of lameness and evidence of splint formation on the forelimbs of the horses were noted.

**Results** A total of 18 horses from Group A exhibited lameness at some point during the summer grazing season while 7 horses from Group B were adjudged to have been lame during the same timeframe (Fig 1). While not all animals that had been lame were to develop splints, 9 horses on farm A had forelimb splints at the end of the summer grazing season and 2 horses from farm B had developed forelimb splints. Moreover, of the animals with splints, one individual from Group B and two horses from Group A did not exhibit lameness. Independent Samples *t-test* analysis of the data revealed that significantly more horses were lame on farm A than farm B ($t = 2.54$, $d.f. = 49$, $P < 0.05$) and significantly more horses also developed forelimb splints on farm A that farm B ($t = 2.17$, d.f. = 49, $P < 0.05$) during the summer grazing season.

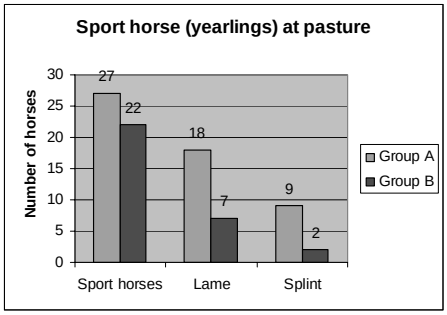


**Figure 1** Total number of horses, horses that were lame and horses that developed forelimb splints while as pasture.

**Conclusions** The findings suggest that rotating yearling sport horses through smaller paddocks during the summer grazing season may be a useful management strategy for the prevention of lameness and/or splint formation in young horses. Yearling sport horses may be more at risk of forelimb injury/splint formation when exposed to expansive grazing facilities, perhaps as a result of increased motor activity. The use of smaller/more confined grazing paddock areas may assist with avoiding and controlling overly excessive play and motor activity among groups of young horses (geldings). Further investigation in this area of equine husbandry to identify optimal summer grazing strategies for young horses is warranted.

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**032**

# Insulin, metabolites and fatty acids concentrations in blood plasma of horses offered linseed oil in the concentrate

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**Introduction** The most striking effect of effort in horses is an increase in energy requirements covered mainly by intakes of large amounts of cereals. Cereals are high in starch, an easily available carbohydrate. High starch feeding is a well known risk factor for the development of intestinal disorders and injuries such as laminites (Clarke *et al.*, 1990). The use of oil in horses diets is an alternative to reduce cereals incorporation. Corn oil, soja oil or coconut oil are often incorporated owing to their good palatability. The aim of the present work was to propose linseed oil as an alternative for fat supplementation in horses rations.

**Materials and methods** Four adult horses aged 7-10 years and weighing 520±50kg were used in a crossed-over design with 3 months periods. They were fed at maintenance either a control diet or a diet in which linseed oil was incorporated. The diet was made of grass hay and concentrate in equal proportions. Linseed oil was included at a rate of 80g/kg in the concentrate. Blood samples were obtained before the morning meal and then every 30 minutes over a period of 5 hours. Insulin, plasma metabolites and fatty acids from esterified fatty acids were measured in the plasma samples.

**Results** The ether extract contents of the linseed oil and the control concentrates were 96 and 20 g/kg respectively so that the ether extract contents in the diet (hay and concentrate) were 16 and 54 g/kg respectively. There were no significant effects of linseed oil on the average concentration of glucose, non esterified fatty acids and triglycerides in the plasma (Table 1). By contrast, linseed oil reduced to a large extent the average concentration of insulin. The pattern of insulin profile was also significantly affected (Figure 1). The concentrations of most of the individual fatty acids were significantly affected by linseed oil inclusion.

**Table 1** Average concentrations over a six hour period of sampling.

| | Control diet | Linseed oil diet | SEM | P<F |
|---|---|---|---|---|
| Glucose (mmol/l) | 5.15 | 5.21 | 0.08 | NS |
| Non ester. F.A. (mmol/l) | 0.17 | 0.19 | 0.02 | NS |
| Triglycerides (mmol/l) | 0.18 | 0.15 | 0.01 | NS |
| Insulin (µIU/ml) | 31.30 | 17.00 | 3.39 | 0.005 |
| C14:0 mg/100ml | 0.40 | 0.27 | 0.02 | 0.001 |
| C16:0 | 15.86 | 15.97 | 0.68 | NS |
| C16:1 n-7 | 1.39 | 0.76 | 0.04 | 0.001 |
| C18:0 | 20.70 | 27.89 | 1.06 | 0.001 |
| C18:1 n-7 + n-9 | 12.93 | 11.38 | 1.22 | 0.05 |
| C18:2 n-6 | 63.26 | 83.98 | 3.73 | 0.001 |
| C18:3 n-3 | 2.08 | 3.61 | 0.40 | 0.001 |
| C20:0 | 0.60 | 0.75 | 0.05 | 0.001 |
| C20:4 n-6 | 1.13 | 0.94 | 0.09 | 0.001 |

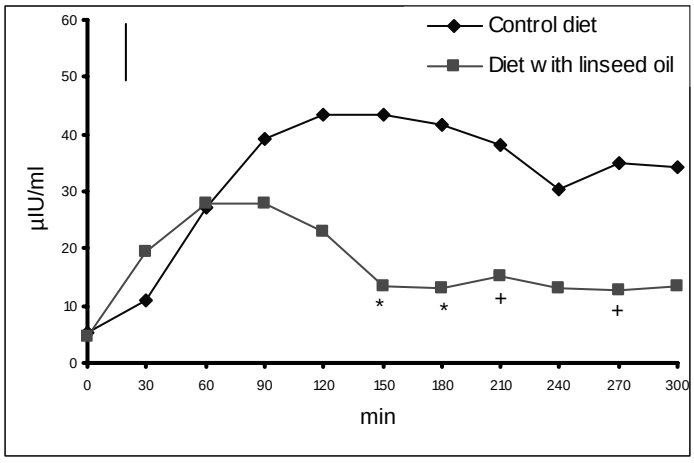


**Figure 1** Changes in insulin

**Conclusion** Although the data were obtained on 4 horses only, the most interesting effect of linseed oil was a reduction of the post prandial rise of insulin. Implications could be expected in the prevention of developmental diseases and digestive disturbances

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**033**

# The effect of rider gender on performance at international equine eventing competitions

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**Introduction** Analysis of environmental factors within equine sporting competition is critical to understanding potential effects on performance. If such effects are occurring they need careful consideration within any performance assessment mechanisms that are subsequently undertaken on the data. Such effects may have implications for competitor's selectors and trainers. Additionally and importantly if the performance data is being related to breeding information, these environmental effects may be playing a significant role in our subsequent judgements and predictions within breed improvement models.

Within competitive sport almost exclusively males and females compete against only their own genders, the reasons for this have been debated at length (Tännsjö and Tamburrin, 2000). However the equestrian disciplines (predominately dressage, showjumping and eventing) are exclusively sporting environments where men and women compete on an equal non-handicapped basis. This is apparent from grass roots level through to Olympic and World Championship level. This study investigates differences between male and female competition results for eventing competition (CIC*** level, the highest level of international one day eventing competition).

**Materials and methods** Data was collected from all CIC*** level competitions run within the UK during 2006, in total four separate events. Within this study only data from competitors completing competition was analysised. Previous work (Whitaker and Hill 2005) has indicated that non-completion data within eventing competition can be regarded as completely missing at random. The dependant variable within the analysis was defined as gender, independent variables (phases of competition) were returned as penalty scores for, dressage, show jumping (both jumping and time), cross country (both jumping and time) as well as final rank in competition. Descriptive statistics and measures of dispersion were returned. Independent sample student *t* test were undertaken on the data sets.

**Results** In total 348 competitors completed competition (218 ♀ 130 ♂). Table one returns descriptive and dispersion statistics for the data. Although the mean male rider is finishing approximately three places higher than the mean female rider, no overall significance (P=0.185) was observed between gender and final rank in competition. However it was noted that for the independent variable cross country time a significant difference (P=0.006) was observed with male riders scoring on average 3.429 penalties lower than females. It was also noted that a trend may be present for show jumping time penalties (P=0.066) with female riders scoring 0.397 fewer penalty points than males.

**Table 1** Descptive and dispersion statitics for rider gender and performance

| | Rider gender | Mean | std.dev | s.e.m |
|---|---|---|---|---|
| Rank in competition | ♀ | 27.615 | 19.093 | 1.293 |
| | ♂ | 24.723 | 20.548 | 1.802 |
| Dressage penalty score | ♀ | 58.625 | 8.174 | 0.554 |
| | ♂ | 58.530 | 6.899 | 0.605 |
| Show jumping jumping penalty score | ♀ | 5.615 | 5.311 | 0.360 |
| | ♂ | 4.930 | 5.256 | 0.463 |
| Show jumping time penalty score | ♀ | 0.688 | 1.817 | 0.123 |
| | ♂ | 1.085 | 2.132 | 0.188 |
| Cross country jumping penalty score | ♀ | 7.431 | 17.632 | 1.194 |
| | ♂ | 5.769 | 14.765 | 1.295 |
| Cross country time penalty score | ♀ | 25.407 | 10.979 | 0.744 |
| | ♂ | 21.978 | 11.259 | 0.988 |

**Conclusions** This study demonstrates that although rider gender is exerting effects within the distinct phases of competition overall it is having no significant effect on final rank in competition. These within phase effects seem to be based around time penalty scores and this may be a observation worthy of further investigation. The study present investigates one level of the sport, run at four locations within the same year. Further work is needed to investigate whether effects are consistent between levels, location and time. If such effects are established they need to be carefully considered in light of any evaluations made on performance.

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**034**

## Observations on the 24 hour activity patterns of stabled horses

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**Introduction** Activity patterns of domesticated animals have largely focussed on hours of daylight and relatively few studies include detailed observations of night time activity. This has the potential to overlook behaviours of significance to the assessment of welfare. For example, stereotypic activities in laboratory mice are largely confined to dark periods, and consequently are not commonly reported by daytime laboratory workers. Use of low light video cameras coupled with infra-red or low intensity lighting now makes observation over entire light-dark cycle practical, whilst minimising disturbance to the sampled population. This paper describes the activity patterns of stabled horses over 24 hour periods. These observations can then be used as baseline for investigating the effects of changes to the stable environment on horses' behaviour and welfare.

**Materials and methods** Data was collected from 10 adult horses (3 mares and 7 geldings; aged between 5 and 17 years of age) housed in Lodden boxes within the same barn. They were fed a concentrate and forage meal at 0800h, a forage meal at 1200h and a further concentrate and forage meal at 1700h each day. Each horses' ration was determined by its maintenance requirements, but on average they received 10.6(±0.5)kg of forage per day, of which 70% was presented in the 1700h meal and the remaining 30% was split equally between the 0800h and 1200h presentations. Forage was presented in conventional haynets. Five horses received haylage as their forage (9.4±0.7kg per day) and were bedded on dust extracted shavings, whilst the remaining 5 horses received hay as their forage (11.8±0.5kg per day) and were housed on straw bedding. Subjects received two hours exercise per day between 0900h and 1100h or 1400h and 1600h, and were not disturbed between 1700h and 0800h. The dark period lasted from approximately 1930h to 0430h. Behaviour was recorded for 24 hours using time-lapse video recorder to minimise disturbance. Low light level cameras were used and stables were illuminated by low intensity red lighting at night. Data collection concentrated on feeding and foraging behaviour, general activity and incidence of any apparently abnormal behaviour, and was scan sampled every 5 minutes for duration of observation. Data were converted to percentage of scans, and the effects of stable management (forage/bedding type) was analysed for observations between 0800h and 1700h (Day) when horses received some human disturbance, and 1700h to 0800h (Night), when human disturbance was minimal. A repeated measure ANOVA was used to assess the effects of forage type on behaviour and intake in day and night periods.

**Results** Horses consumed on average 9.4±3.2 kg of forage per 24 hours which represents 87% of available forage. Horses ate a greater proportion ($P<0.01$) of forage (91%) during the night (6.8±1.3 out of 7.4±1.2kg) compared with 75% during the day (2.6±1.4 out of 3.2 ± 1.5kg). Nearly all hay was consumed at night (97.3% or 7.6 out of 7.7kg) but only 84.9% of haylage (6.1 out of 7.0 kg). During the day observations, a greater proportion of hay (89.2%) was consumed compared with only 59.6% of haylage ($P<0.05$). Over 24 hours, eating forage (32.3%) and standing/resting (26.5%) were the most common activities. Horses also spent 16.1% of scans lying, 12.8% standing alert and 2.9% of scans in bedding directed activities. Other activities made up a small part of horses' day and stereotypic behaviour was rarely recorded (less than 0.5% of scans). Eating concentrate occupied 3.1% of scans and was concentrated in the hour immediately following meal presentations at 0800h and 1700h. Standing alert was most common in the hours preceding feeding time and foraging was observed throughout the day and night, but with peaks following the forage presentations at 1200h and 1700h. Standing dozing was most common during periods of low disturbance, in the early afternoon and at night. Lying was rare during daytime and mainly observed at night particularly between dusk and dawn. Horses with straw/hay spent more scans eating forage (39.2±1.5%) than those with shavings/haylage (25.3±2.1, $p<0.001$). They also performed more drinking (1.2±0.2% vs 0.4±0.2, $p<0.05$) and bedding directed activity (4.3±0.8% vs 1.6±1.1, $p<0.05$). Horses with haylage spent more time standing (45.8±2.1% vs 32.9±1.6%, $p<0.001$), lying (17.7±0.9% vs 15.2±0.7%, $p<0.05$) and eating concentrate (2.0±0.3% vs 4.1±0.6%, $p<0.05$) than horses who received hay.

**Discussion and Conclusion** It is estimated that free ranging horses spend 60% - 70% of their time engaged in foraging activities (Thorne et al 2005). In our study only 40% of scans were engaged in feeding activities including eating forage, eating concentrate and bedding directed activities. This low figure does not cause concern in itself as redirected or abnormal repetitive behaviour was rarely observed. The increase in bedding directed behaviour in horses fed hay with straw bedding compared to those fed haylage with shavings bedding was expected, and although straw ingestion has been associated with an increase in colic in some individuals (Thorne et al 2005), again there was little evidence of actual ingestion of straw in this population.

**Ackowledgements** This project was funded as part of a Higher Education Innovation Fellowship. We would also like to thank the horse owners and University of Lincoln Equine Yard for access to horses and WALTHAM for their advice during the project.

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**035**

# Equine breed registry and type: its influence on performance on young event horse evaluation

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**Introduction** European breeding schemes have for many years routinely conducted evaluation of young (mainly at four to six years of age) sport horse as a part of their integrated breed improvement schemes (Whitaker and Hill, 2005 a). Such an approach has allowed assessment of horses as to their future worth as competition animals and helped to establish the potential genetic worth of animals to the populations. Furthermore such events act as part of the validation mechanism for the establishment of estimated breeding values. The UK has historically suffered from an unstructured and disparate approach to sport horse breeding (Whitaker and Hill 2005 a). However recently (over the last five years) various organisations have introduced competitions aimed at assessment of young sport horses. An example is the British Eventing Breeding Championships established in 2004. Four, five and six year old event horse compete in qualifying competitions and then participate in a one day championship event run in October. This study investigated the effect of both breed type and registry on final rank within competition for six year old horses completing competition in 2004 and 2005 championships.

**Materials and methods** Competition data was collated for all horses completing competition. Previous work (Whitaker and Hill 2005 b) has indicated that within eventing competition non-completion can be classified as completely missing at random. It was therefore considered appropriate not to consider non-completers further. Competition data for individuals was reconciled against information relating to horses breeding (seven types defined) and registration (10 types defined). Distribution patterns were established for the data sets. Data was additionally analysed via a two way analysis of variance with regard to breed type, registration and breed type, registration interaction. Further analysis was conducted via two backward linear regression models (A and B) to establish r squared for the dependant variable (final rank in competition), independent variables where described as breed type in model A and breed registration in model B

**Results** In total 85 horses completed competition. Figure 1 and 2 return the respective percentages for breed type and breed registration for horses completing competition. Results of Analysis of variance from this investigation shows that no significant effect of breed type (P=0.204), breed registration (P=0.832) or breed type registration interaction (P=0.542) is apparent are returned for in table one. The r squared returned for model A, breed type was 0.024 and for Model B breed registration 0.001

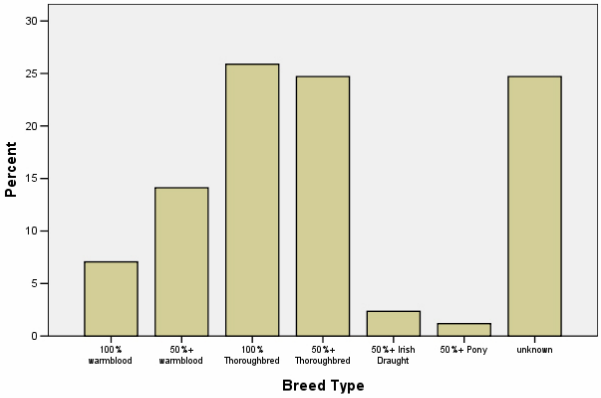


Figure 1. Completing horses by breed type

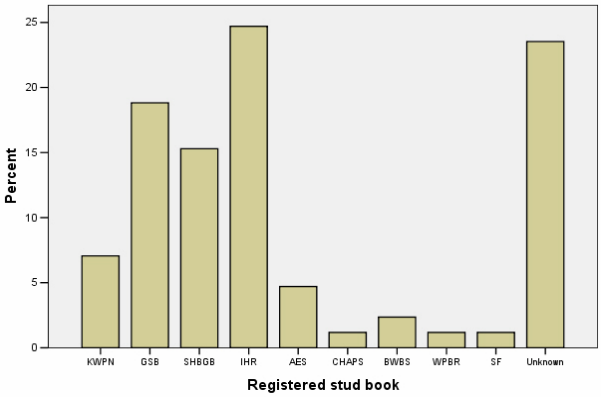


Figure 2. Completing horse by registered stud book

**Conclusions** Breed type, registry or the interaction of these two factors are not exerting a significant effect on final rank in competition. Additionally the r squared returned for each of these factors is negligible. It should be noted that the percentage of unknown horses both with regard to breed type and registration is approximately 25% of the population. Such a percentage is concerning considering the nature of the competition involved. The study was conducted on a small number of horses and any effects described have to take this into consideration.

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**036**

# Factors influencing the demand for British bred horses

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**Introduction** The Sport Horse breeding industry in Great Britain has been in decline for many years. Once, the British were in the vanguard of the worldwide Sport Horse breeding industry, alongside a buoyant Thoroughbred breeding industry. However, in recent years Sport Horse breeding has floundered, with many competitive dressage and show jumping horses being purchased from overseas. (BHIC / Defra 2005) Research has been carried out to identify trends in Thoroughbred auction sales (Buzby and Jessup 1994, Robbins and Kennedy 2001), and Sport Horse auction sales (Hennessey and Quinn 2006). The British Equestrian Federation (BEF), through *British Breeding*, commissioned this research firstly to ascertain the demand for British bred horses and identify any trends contained within this demand, and secondly to obtain a snapshot of the current horse buying market place.

**Materials and methods** An online questionnaire was utilised within the study (a paper based version was also available). The questionnaire was available between November 2005 and January 2006. A number of statistical tools were used to analyse the results. These included chi-squared analysis ($\chi^2$), ANOVA, the student's t-test and a posteriori test, the Scheffé test (Kirk 1995). Where applicable the probability levels were P=<0.05. Ten semi-structured interviews were also completed, either face to face with interviewees or over the telephone, in January and February 2006. The interviews focused on a number of themes that were identified through discussion with key industry representatives and as a result of preliminary data analysis from the questionnaires.

**Results** A total of 1,308 responses to the questionnaire were received. Of those 1,280 were from respondents based in Great Britain, and these were used to obtain the results of the study. Significant differences were found between the average prices paid for horses when the horses purchased were considered by area of breeding, through the use of a single factor ANOVA, followed by Scheffe's multiple comparison test (P=0.01 for ANOVA, P=0.05 for Scheffé). These differences are shown below in Table 1. Further significant differences were found when other attributes were analysed. These include height, age, primary activity of buyer (e.g. Amateur or Professional Competitor) and the number of horses viewed. Respondents were questioned about their attitudes to British bred horses. 70% of respondents stated that they would buy a British bred horse, with a further 28% of respondents not concerned about the nationality of the horse they buy. 13% of respondents felt foreign bred horses were better than British bred horses, while 23% of respondents felt that British bred horses were of a better quality than foreign bred horses. 62% of respondents would buy a British bred horse because they wanted to support the British horse industry. The questionnaire also asked respondents about their purchasing behaviour when buying horses. Statistically significant differences were found when considering the distance respondents were willing to travel to look at horses. More respondents with the primary activity of Breeding, Dressage or Show Jumping were willing to travel abroad than expected, while less respondents with the primary activity of Eventing or Recreation were willing to travel abroad than expected ($\chi^2$ = 1.56E-31). Of the respondents 60% researched the history of the last horse they bought, while 73% of respondents had some form of advice or assistance when they purchased their last horse.

**Table 1** Comparison of average price paid (all horses)

| Area of breeding | Mean (£) | Min (£) | Max (£) | n |
|---|---|---|---|---|
| Great Britain | 2,662 | 0 | 40,000 | 604 |
| Ireland | 3,918 | 0 | 25,000 | 203 |
| Rest of Europe | 7,652 | 1,000 | 50,000 | 156 |
| Other | 3,396 | 500 | 7,000 | 12 |
| Unknown breeding | 2,990 | 0 | 50,000 | 52 |

**Conclusion** A number of factors were found to influence the demand for British bred horses, including the perceptions respondents held about these horses. Country and area of breeding were shown to highly influence the price paid for a horse. Respondents with the primary activities of Breeding, Dressage and Show Jumping were found to be more likely to purchase a horse from abroad than expected. The findings of this study imply that the British horse breeding industry could improve its market share through a deeper understanding of the needs and expectations of 21st century consumers.

**Acknowledgements** Funding for this study was provided by the BEF through *British Breeding*.

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**037**

# Investigating the effect of feeding space on aggression, feeding behaviour and production

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**Introduction** Promoting feed intake of lactating dairy cattle (particularly those in early lactation) is critical in terms of improving milk production, health, body condition and welfare of the animals (Grant and Albright, 1995). Therefore a good food supply is extremely important to the modern, high producing dairy cow. Feeder design and stocking density can have a major impact on feed intake and aggressive behaviour; therefore it is an important consideration when designing housing and managing livestock. The aim of the study was to investigate if increasing space allowance at the feed-face would reduce the frequency of aggressive interactions and competition. These changes in spacing may also lead to an increase in feeding activity in the time after provision of fresh feed.

**Materials and methods** Forty-five lactating Holstein Friesian cows were used in the study, and all cows were multiparous (parity = 4 $\pm$ 1.9; mean $\pm$ SD). Cows were allocated to 3 groups of 15 animals, and were balanced across the groups for age, stage of lactation and milk production. The groups were housed in 3 adjacent pens, and were fed a Total Mixed Ration (TMR) provided once daily at approximately 09.00. The TMR consisted of grass silage (60%), mix (11%) grain maize (17 %), lupins (5%) alkalage (6%), and minerals (1%). Cows were fed using a post and rail barrier, and were milked twice daily. Each of the 3 groups was exposed to 3 different feeding space allowances, using a Latin square design. The space allowances (treatments) were 0.35, 0.69 and 1.04m per cow and were chosen to represent low, standard, and high allowances. Each treatment lasted for a period of 9 days, with the first 2 days acting as a habituation period. Feeding, and aggressive behaviours, feed intake and milk yield were recorded. All behaviours were continuously monitored using video cameras, and cows were identified using unique alphanumeric symbols. The footage analysed was from 60 minutes after the delivery of fresh feed and 60 minutes after returning from milking, as this is generally recognised to be the period of highest competition. ANOVA was used to assess effects of treatment on behaviour and production. REML was used to analyse length of feeding bouts with the treatment as a fixed effect and cow and groups as random effects.

**Results** The length of feeding bouts decreased ($P<0.05$), and the number of bouts increased ($P<0.001$) as the space allowance increased (Table 1). Also, the number of aggressive interactions increased as the space allowance decreased ($P<0.001$) and the number of times individuals were displaced from the feeding area also increased as the space allowance decreased ($P<0.05$). There was a significant difference between feed intakes ($P<0.05$) and milk yields between treatments ($P<0.05$). For the low and standard space allowances the combined milk yields were similar (90.52kg and 90.72kg respectively, Table 2). Therefore it is felt that differences between milk yields and feed intake are possibly due to biological variation and it would require a longer period of observation to fully interpret the results.

| Measures** | Feed-face space per cow | | | L.S.D. | P |
|---|---|---|---|---|---|
| | 0.35m | 0.69m | 1.04m | | |
| No. of bouts | 1.994 | 1.987 | 2.532 | 0.249 | < 0.001 |
| Length of bouts (s) | 1285 | 1146 | 1036 | 82.18 | 0.035 |
| All aggressive interactions | 0.874 | 0.582 | 0.305 | 0.266 | < 0.001 |
| Displacements | 0.409 | 0.360 | 0.159 | 0.202 | 0.037 |

**Table 1** Measures of feeding and aggressive behaviour*

* Means calculated for 7d per treatment
** Behavioural data from 2 hours/day

| Measures | Feed-face space per cow | | | S.E.D. | P |
|---|---|---|---|---|---|
| | 0.35m | 0.69m | 1.04m | | |
| Milk yield* | 90.52 | 90.72 | 88.80 | 0.839 | 0.046 |
| Feed intake** | 1911.6 | 1815.8 | 1986.5 | 15.56 | 0.016 |
| Weight | 652.0 | 653.9 | 648.7 | 4.54 | 0.509 |

**Table 2** Measures of milk yields, feed intakes & weight.

* Mean total of individual yield over 3 day period
** Mean total of feed intake for all animals in group

**Conclusion** As the space allowance increased the overall number of aggressive interactions declined, as did the number of displacements from the feed-face. Therefore, increasing space for feeding should allow undisturbed, normal feeding patterns. This is likely to be as a result of a lower level of competition. As the length of feed-face increased the number of bouts increased and length of bouts decreased. This suggests that under free feeding conditions cows appear to prefer to eat in frequent, short bouts, which supports the findings of Grant and Albright (1995). There was no consistent relationship between feed intake, milk yield and space allowance. Over stocking at the feed-face should be avoided to increase activity and reduce competition. It is recommended that feed should be available when cows desire to eat, e.g. at sunrise or after milking, to ensure that feeding behaviour and total feed intake are optimised. Future research should consider the long term effects of over crowding and competition on dry matter intake (DMI), milk production, and health.

**Acknowledgements** Financial support of SEERAD and technical support of farm staff at SAC Dairy Research Centre, Crichton Royal Farm are gratefully acknowledged.

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**038**

# Methods for assessing sociability of dairy cows

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**Introduction** Sociability refers to the extent to which animals seek social companionship. Individual differences in social behaviour exist between animals. It is important to measure individual social motivation in order to develop suitable temperament scores that can be used in future breeding programmes or as part of welfare assessment schemes. Runway tests have been shown as reliable methods of measuring sociability in animals (Birds Mills & Faure, 1991; Sheep Sibbald *et al.* 2005). The aim was to develop a suitable test that could be used to measure sociability of cows in a commercial situation. The following questions were addressed i) How repeatable is the runway test when carried out on dairy cattle, ii) Does a cow's performance in a runway test correlate with social behaviour within a group house setting?

**Materials and methods** Fifty-four Holstein-Friesian cows from lactation 1 to 10 were used. Behavioural scans to assess sociability in a housed group were carried out on the whole group with more detailed social behaviour scans and a runway test carried out on 46 individuals.
The runway test was repeated three times on each animal once a week for three weeks. The group of 46 cows was split into 3 random groups of 15, 15 and 16 cows each week, therefore, each time an animal was tested, it was tested with a different group of animals. Groups were balanced for lactation (1,2,3,4,5+). On test days, a group of animals were moved to a holding pen. In a random order, each cow was removed from its test group and herded up to the start line of an 18m corridor by the same experimenter. The cow was held behind a gate, allowed to settle for 30s, and then released to allow her to move towards her group mates. The maximum time for each test was 300s. All tests were recorded by digital camcorder. Analysis of videos included the latency to reach a 5m and 2m (close zone) distance from group mates. The time spent within these areas was taken as measurements of social motivation.
General observational scans recording place, posture and behaviour of each cow in the whole herd were carried out at 20 minute intervals for a total of 9 three hour periods. During these scans, each of the 46 cows from the focal group were observed and the place, posture, behaviour, position, identities and distances away from the 2 nearest neighbours of each focal animal recorded. All scans were carried out by the same observer. Comparative data were analysed using GLMM procedures in Genstat 8.

**Results** Repeatability of latency to reach the 5m line over the three tests was moderately high (0.54) and the most repeatable measure taken. For each focal animal, a tally of presence and absence at the feedface during peak feeding time as well as the number of times observed feeding at the end of the feedface was converted to a proportion from the total number of sample points. Cows observed with neighbours at a distance less then 1 meter away were considered "near" neighbours and greater than 1m were labelled "far" neighbours. As latency to 5m line increases the proportion of observations of having two near neighbours decreases, ($P<0.01$), and the proportion of observations of having two neighbours far away increases ($P<0.05$).

**Table 1** Correlation ($s_r$) table of 5m latencies and social behaviour from scans

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| 1 5m Latency week 1 | - | | | | | | |
| 2 5m Latency week 2 | 0.600 | | | | | | |
| 3 5m Latency week 3 | 0.478 | 0.624 | | | | | |
| 4 Presence at Feed face | -0.141 | *-0.367* | -0.070 | | | | |
| 5 Absence at Feed face | 0.160 | *0.351* | 0.134 | -0.788 | | | |
| 6 End of Feed face | 0.181 | 0.211 | 0.128 | -0.459 | 0.655 | | |
| 7 Two near neighbours | -0.247 | -0.384 | -0.157 | 0.816 | -0.750 | -0.695 | |
| 8 Two Far neighbours | 0.170 | *0.340* | 0.095 | -0.736 | 0.492 | 0.143 | -0.807 |

Column numbers in the top row correspond to the numbered variables in the first column. *$P<0.05$* or $P<0.01$

**Conclusions** The runway test showed good repeatability therefore was considered a good test of social motivation in cows. As the latency to the 5m line increased the proportion of observations of having two near neighbours decreased and the proportion of observations of having two neighbours far away increased. Therefore, cows with low social motivation in the runway test tended to be observed further away from other cows in their housing area. Observations of distance from neighbours could be used to assess social motivation in on-farm studies.

**Acknowledgements** The authors acknowledge the support of DEFRA, NMR, CIS, Genus, Cogent, HUK, BOCM Pauls, Dartington Cattle Breeding Trust, Avoncroft Sires and RSPCA through a LINK Programme and SEERAD.

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039

# Posture changing behaviour as an indicator of floor comfort in finishing pigs

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**Introduction** The ease with which posture changing occurs in pigs has welfare relevance since the duration of lying down and standing up behaviour may indicate the adequacy of floor type to give security of movement, and/or the mobility of animals after resting on floors differing in comfort. Standing and lying behaviours consist of a predictable sequence of movements, with stages between movements when the animal can pause in a relatively stable transition state. In sows, it has been suggested that the duration of one or more of these stages might be sensitive to both animal and environmental factors (Marchant and Broom, 1996); however whether this is the case in finishing pigs is less certain. The aim of this study was therefore to evaluate behaviours indicative of both the physical comfort of different types of floors and security of movement in finishing pigs.

**Materials and methods** Groups of 8 Large White x Landrace pigs were allocated at ~80 kg to pens on each of three different floor treatments: part-slatted concrete (PS), fully-slatted concrete (FS) and deep straw bedding (ST). Four replicate pens were studied per floor treatment at approximately 100 kg liveweight over a period of four weeks. Pigs were assessed for the presence of leg lesions, using a pre-determined scale ranging from 0 (no graze) to 5 (open wound). The presence and severity of adventitious bursitis was also visually assessed (0-no bursitis to 5-large eroded bursae with infection). A portable digital camera was used to take real time records of the pigs in each pen during posture changing between standing and lying (five stages: kneel, pause, shoulder on floor, pause, lower hindquarters), and lying and standing (three stages: sit, pause, lift hindquarters to stand). The film was subsequently analysed with Windows Media Payer, allowing 0.04s time frames to be analysed. Pen means were calculated for the times for each stage of the posture transition, the total time for the completion of the transition, and also for leg lesion and bursitis scores. The effects of floor type on these timings were analysed by ANOVA with pen as the statistical unit. Pearson's correlations were used to analyse the relationship between lesions and posture changing duration.

**Results** A significant effect of flooring type was seen in the total amount of time taken to change posture from a standing position to a lying position, and in the timings of three individual stages (Table 1). There were no significant effects of floor type on the total amount of time taken to change from lying to standing, although stage 3 of this transition was significantly longer for FS pigs than ST pigs (2.00 v. 1.01s, S.E.M. 0.142, P<0.01).

**Table 1** The effects of floor type on the timings (sec) for each of the five stages comprising the transition from standing to lying

| Transition stage | FS | ST | PS | S.E.M | P |
|---|---|---|---|---|---|
| Stage 1 | 1.28[a] | 0.72[b] | 1.23[a] | 0.062 | *** |
| Stage 2 | 0.18 | 0.40 | 0.27 | 0.131 | ns |
| Stage 3 | 1.33[a] | 0.70[b] | 1.02[a,b] | 0.121 | * |
| Stage 4 | 0.08 | 0.16 | 0.08 | 0.031 | ns |
| Stage 5 | 2.57[a] | 1.09[b] | 1.73[c] | 0.143 | *** |
| Total time | 5.43[a] | 3.06[b] | 4.34[c] | 0.276 | *** |

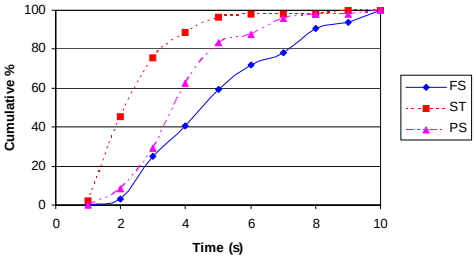


**Figure 1** The effects of floor type on the cumulative % of pigs completing the transition from standing to lying within different times from the start

The cumulative frequency of the total time taken to lie down for each floor type was plotted for the standing to lying transition (Fig 1). On ST floors, the great majority of pigs completed the transition within five seconds of initiation; in contrast, only 60% of FS pigs did so. Lower leg (r=0.863, P<0.01) and claw lesions (r=0.699, P=0.011) and hock bursitis scores (r=0.589, P<0.05) were moderately but significantly correlated with the total amount of time taken for the standing to lying transition. Few significant correlations with physical injury were observed for the lying to standing transition.

**Conclusions** The duration of the standing to lying transition gave a measure which differentiated between floors giving different levels of support and cushioning. The duration of the lying to standing transition was much less informative, which may reflect the different types of standing seen in normal standing and as an alarm response (Marchant and Broom, 1996). The speedy transition from standing to lying appears, from correlations with injury, to reflect good control as a result of a cushioned floor with good grip. The duration of the standing to lying transition therefore may be a useful measure of the adequacy of flooring type for pigs.

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**040**

# Can linear enamel hypoplasia be used as a novel welfare indicator in pigs?

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**Introduction** Linear enamel hypoplasia (LEH) produces abnormal incremental lines or depressions in teeth enamel caused by a deficiency in the growth of calcified tissue. The depth and width of the LEH events can indicate the severity and duration of a stressful life event. LEH can be used to investigate developmental stress, as it will occur when the individual is unable to cope with environmental stressors during development, such as food shortage, pathological challenge or other stressful events. The LEH technique has been used by zoo-archaeologists to obtain information on early domestication and husbandry practices in ancient populations of pigs, where high levels of LEH have been found to correspond to birth and weaning. The objective of this work was to investigate if LEH was present in modern pigs and whether it could be used as a welfare indicator.

**Materials and methods** 180 lower mandibles were collected from finished pigs (between 6 and 7 months of age) from six English breeder-finisher farms, with 30 from each farm. There were two groups of three farms; one group (HIGH) had significantly higher scores (Z-value = -1.984*♦, Mann-Whitney U Test) than the other group (LOW) when the rearing conditions and the animals were assessed using an adapted Animal Needs Index (aANI) (Bartussek, 2001). The aANI was based on a system developed in Austria that uses resource- and animal-based methods to assess on-farm welfare; categories included space allowance, cleanliness, condition of pigs' skin, feet and joints. The hypothesis was that the occurrence of LEH would be higher in the LOW group, as they would have been exposed to poor rearing conditions.

Within this experiment, the number of LEH events was recorded on the lingual surface of the first molar (M1), which is the only molar erupted at this age (Dobney and Ervynck, 1998). The events were recorded on the anterior (a) and posterior (b) cusp of the right and left M1, and as each cusp develops independently each can be analysed for LEH separately. This meant that information from 4 cusps was recorded.

A Mann Whitney U Test was used to compare the HIGH and LOW group, and all the results were subjected to sequential Bonferroni correction.

**Results** LEH did occur in modern domestic pigs and there was higher occurrence of LEH events than in archaeological samples from pigs. For example, Dobney *et al*. (2002) reported the mean number of LEH observations per cusp ranged between 0.06 and 0.63 for M1a, and 0.09 and 0.51 for M1b for all populations. In this experiment, the mean of LEH observations per cusp ranged from 1.93 to 2.41, which is higher.

For the mean number of events per cusp, the HIGH group had a significantly higher number of LEH events than the LOW group on each cusp investigated (see Table 1). This meant the hypothesis was rejected.

**Table 1** The mean number of LEH events for the four cusps.

| Cusp | n^ | Mean Number of Events ± Standard Deviation | | Z-value |
|---|---|---|---|---|
| | | HIGH group | LOW group | (Mann-Whitney U Test) |
| Left M1a | 173 | 2.55 ± 0.93 | 1.94 ± 0.78 | -4.19***♦ |
| Left M1b | 171 | 2.30 ± 0.87 | 1.98 ± 0.88 | -2.49*♦ |
| Right M1a | 172 | 2.41 ± 0.90 | 2.02 ± 0.90 | -2.75**♦ |
| Right M1b | 173 | 2.39 ± 0.84 | 1.93 ± 0.86 | -3.43***♦ |

(M1 = First molar, a = anterior cusp, b = posterior cusp, ^ = number was reduced due to damage to mandible,
* = P<0.05, ** = P<0.005, ***= P<0.001, ♦ = significant after sequential Bonferroni correction.)

**Conclusions**
These results did not support the hypothesis that the pigs reared in poor conditions (LOW group) would have a higher number of LEH events, since it was found that pigs in the HIGH group had significantly more events. These results are difficult to explain, as so little is known about the type, duration and severity of stress that is needed to cause LEH events in modern pigs. It is worth considering the massive changes that have occur in the domestic pigs in the last few centuries, and the increased growth rate in modern pigs is likely to result in greater physiological demands, which could lead to developmental instability and an increased occurrence of LEH events. More information is needed on the causes of LEH in modern pigs, and also the physiological demands that could affect teeth development before LEH can be further investigated as a welfare indicator.

**Acknowledgements** E Genever's PhD studentship was funded by BBSRC.

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**041**

# The use of enrichment objects by individual pigs in commercial groups

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**Introduction** The provision of environmental enrichment for pigs is a legal requirement within the EU. However, there is debate as to what constitutes suitable enrichment in commercial environments where straw cannot be used. The objective of this study was to investigate the use of objects by pigs under UK commercial conditions, and the extent to which individual pigs used, or were prevented from using, the enrichment provided.

**Materials and methods** Three hundred and sixty pigs (Large White × Landrace) were selected at 55 ± 5 kg and housed at commercial stocking densities in groups of 12 until they reached an average liveweight of 90 kg. A randomised block design with six replicates of five treatments was used (total 72 pigs per treatment). The five treatments were: a fully-bedded straw pen (ST – positive control), a BiteRite which is a commercially available enrichment object with chewable plastic "tails" (BR), a substrate dispenser filled with straw (SD), a rootable feed dispenser containing flavoured feed (FD) and a chewable liquid dispenser containing flavoured water (LD), (Van de Weerd *et al*, 2006). With the exception of the ST pens all pens were part-slatted. The pigs were continuously videoed for the duration of the study (seven weeks). The videos from the first full day (week 1) and one day in weeks 3 and 7 were analysed, between 09:30 and 20:30 h, using scan time sampling. Prior to these observation periods the pigs were individually marked using spray marker. The behaviour of each pig was recorded at six minute intervals with only the enrichment directed behaviour reported here. Data were analysed using ANOVA, with pen as the unit, and Spearman Rho correlations ($r_s$). All analyses were performed using SPSS version 11.0.

**Results** There was a significant effect of treatment ($p<0.001$) on the proportion of pigs using the enrichment (Table 1). All pigs exposed to ST were observed to use the enrichment at some time during each observation period whereas out of the 288 pigs exposed to BR, SD, FD and LD, 154 were never observed using the enrichment. There were significant correlations between enrichment use by individual pigs for the three observation periods suggesting that the same pigs displayed enrichment directed behaviours consistently over the seven week period (Table 2). Restricting the data to only those pigs observed to use the enrichment, the mean proportion of scans where the enrichment was used remained relatively constant over the seven week period (0.07, 0.07 and 0.06 for weeks 1, 3 and 7 respectively, s.e.d. = 0.006) suggesting that pigs which did use the enrichment did so consistently over a prolonged period of time. The design of the enrichment influenced the maximum number of pigs observed to use the enrichment at any one time (max. number = 11, 4, 5, 4 and 3, for ST, BR, SD, FD and LD respectively). More than one pig was observed using the enrichment at the same time for 0.48 of scans where the enrichment was used, however, there was a significant effect of treatment with pigs exposed to ST using the enrichment synchronously with other pigs more than those exposed to other treatments (mean proportion = 0.67, 0.21, 0.38, 0.28 and 0.14 for ST, BR, SD, FD and LD, s.e.d. = 0.052, $p<0.001$).

**Table 1** Effect of enrichment on the proportion of pigs in a group observed using the object during that period

| | Enrichment | | | | | | |
|---|---|---|---|---|---|---|---|
| Week | ST | BR | SD | FD | LD | s.e.d. | Sig. |
| 1 | $1.00^{c}$ | $0.35^{a}$ | $0.46^{b}$ | $0.38^{ab}$ | $0.29^{a}$ | 0.042 | <0.001 |
| 3 | $1.00^{c}$ | $0.37^{ab}$ | $0.50^{b}$ | $0.44^{b}$ | $0.24^{a}$ | 0.065 | <0.001 |
| 7 | $1.00^{d}$ | $0.31^{ab}$ | $0.46^{bc}$ | $0.51^{c}$ | $0.14^{a}$ | 0.081 | <0.001 |

$^{abcbd}$Means with different superscripts, within rows, differ significantly ($p<0.05$)

**Table 2** Effect of enrichment on the correlation of individuals' object use over three separate observation periods ($r_s$)

| | Enrichment | | | | |
|---|---|---|---|---|---|
| Week | ST | BR | SD | FD | LD |
| 1 v 3 | 0.315** | 0.841*** | 0.754*** | 0.838*** | 0.590*** |
| 3 v 7 | 0.294* | 0.805*** | 0.816*** | 0.857*** | 0.169 |
| 1 v 7 | 0.049 | 0.902*** | 0.797*** | 0.847*** | 0.673*** |

* $p<0.05$, ** $p<0.01$, *** $p<0.001$

**Conclusions** In this study, the form of enrichment provided had a significant impact on the extent to which it was used by pigs within commercial groups, with many pigs never observed to be using the enrichment despite having access to it. The design of the enrichment also influenced the number of pigs seen to use it synchronously at any given time. Given that all pigs housed in fully-bedded straw pens used the straw, often synchronously, this poses questions as to whether, for other treatments, the enrichment object:group size ratio, or stimulus properties of the object, were sufficient to meet the needs of the group as a whole or the needs of the individuals within the group.

**Acknowledgements** This project used video tapes obtained as part of a Defra funded project (AW0124).
**Reference** Van de Weerd, H A, Docking, C M, Day, J E L, Breuer, K and Edwards, S A (2006). Effects of species-relevant environmental enrichment on the behaviour and productivity of finishing pigs. *Applied Animal Behaviour Science* **99**: 230-247.

042

# Behavioural consequences of the genetic selection for health and fertility or 'robustness' in dairy cows: responsiveness to novelty and human interaction

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**Introduction** Modern dairy cows appear to be less 'robust' or adaptable than in the past, as typified by high involuntary culling and poorer health. This could be addressed by the use of broader breeding goals and breeding indices that include traits that are likely to improve fertility and health. Body energy balance and growth rate in the first lactation are traits that are likely to underlie health and fertility and have been included in an index of robustness (Wall et al., 2006). However, we need to ensure that the inclusion of these new traits does not have any unforeseen effects on animal behaviour, which may reduce the welfare of the individual or the group she is housed in. Selection to improve body condition may mean that the animals seek increased access to feed. It may be hypothesised that this could lead them to be more aggressive at the feed-trough. Alternatively, they may increase their feed intake by becoming unresponsive to the social and physical environment, and feeding outwith the peak feeding period. This could mean that they become less responsive to their environment and possibly become harder to handle. The aim of this experiment was to compare the behaviour of daughters of bulls which scored high on the functionality sub-index of the robustness index ('high functionality' bulls) with daughters of bulls which scored low ('low functionality' bulls) on this sub-index. Specifically, we investigated the responsiveness to the environment, by assessing reactions to a novel object and to an unfamiliar person.

**Materials and methods** Thirty-two commercial dairy farms were identified with at least 8 heifer daughters of high-functionality bulls (H) and 8 daughters of low-functionality bulls (L). Farm visits were carried out during the winter housing period using a team of four observers, with two people visiting any one farm. Heifers were used as the subjects of the study to avoid bias that might be created by differential culling of low functionality vs high functionality animals. To assess the responsiveness to a novel object, a test (NOT) was used in which two black and yellow striped boards (60 x 30 cm) were fixed at cow head-height in the passageway leading from the milking parlour. Fear-related and investigative responses were each scored separately on ordinal scales. Responsiveness to humans was assessed using a flight distance measure and a qualitative assessment of the cow's temperament (HIT). This was recorded using a visual analogue scale for four expressive terms ('at ease', 'attentive', 'nervous' and 'social'). The quality of the stockhandling was also scored for each farm visited. REML models were used to analyse the data.

**Results** Analysis of the behavioural variables from the NOT showed that H heifers displayed significantly more investigative responses towards the novel object than the L heifers ($W_1$=5.01; P<0.05; Figure 1). There was no difference between the groups in the level of fear-related responses shown to the novel object ($W_1$=1.18; P=0.205). Heifers that displayed highly reactive fear responses showed a low level of investigative response to the novel object (r=-0.290; P<0.001). There was no difference between H and L heifers in the measure of flight distance ($W_1$=1.85; P=0.173), however, heifers reared in pens as calves were more approachable than those reared in groups ($W_2$=16.80; P<0.001). There was no difference between H and L heifers in their scores for the qualitative terms (P>0.05 for all).

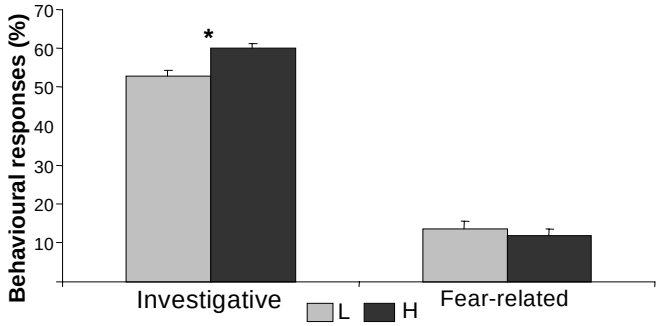


**Figure 1** Graph showing investigative and fear-related responses to the novel object. Mean behavioural scores as a percentage of total possible scores on the ordinal scales are shown.

**Conclusions** Daughters of sires scoring high on the functionality sub-index showed more positive responses to environmental challenges involving both unfamiliar humans and novel objects. This may indicate that they are more capable of coping with changes in their environment, and that selection for robustness will not reduce responsiveness to human handling and to the physical environment in dairy cattle.

**Acknowledgements** This project was funded by Defra, Avoncroft sires, BOCMPauls, CIS, Cogent, Dartington Cattle Breeding Trust, Genus, HolsteinUK, NMR, RSPCA and SEERAD through the LINK Sustainable Livestock Production Programme.

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**043**

# Responses of positively handled calves to human interactions and disbudding

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**Introduction** Cattle have an innate fear of humans that may be reinforced during the potentially painful routine husbandry procedures performed by humans during the rearing of young calves. This fear may be reduced by positive handling of calves early in the rearing period (Krohn *et al.,* 2001) and by use of calming devices such as blindfolds (Mitchell *et al.*, 2004). Mitchell *et al,* (2004) found that blindfolding cattle during restraint and simulated husbandry procedures reduced struggling by 44% and decreased heart rate compared to control cattle with unrestricted vision. However, this study did not test the effectiveness of the blindfold during actual invasive procedures. The aim of the present study was to investigate whether blindfolding during disbudding and positive handling of dairy calves would influence the response of calves to human interactions.

**Materials and method** Twenty seven female Holstein Friesian dairy calves were assigned to either Group C: control group (n=14) or Group H: positively handled group (n=13) at 1 day of age. All calves were housed in individual pens (1.8 m x 0.8 m) bedded with straw and received milk replacer at 0700 h and 1600 h until weaning at 50 (± 0.5) days of age. Three times a week between 0900 h and 1200 h H calves were softly talked to, patted, stroked and brushed with a soft, rubber grooming glove all over the body for six minutes (similar to Krohn *et al*., 2001) until weaning. C calves received routine human contact consistent with good husbandry practice with no additional interactions. C and H calves were further divided into treatment groups for disbudding at 30 (± 1.8) days of age; Group B: disbudded with a blindfold or Group N: disbudded without a blindfold. Immediately before disbudding a 30 x 16 cm blindfold was fitted to calves in group B. All calves were disbudded under local anaesthetic by the same operator. During this procedure a video recorder was used to record calf behaviours. To investigate the fearfulness of calves towards humans and a novel object, willingness to approach tests were carried out at 52 (± 0.5) days of age for 3 minutes towards a familiar person, an unfamiliar person and a beachball. Following this the flight distance (the distance between the person and the calf before the calf retreated) of each calf was measured. Flight distance was measured again at 71 (± 0.4) days of age. Data were analysed using 2-way ANOVA (GenStat version 9, Lawes Agricultural Trust, Rothamsted Experimental Station).

**Results** Time taken to disbud B calves was almost half that taken to disbud N calves (34.4 ± 2.5 versus 66.2 ± 11.4 seconds, respectively; ($P$<0.005)). This finding was associated with a tendency ($P$=0.10) for B calves to show fewer kicking behaviours during disbudding compared to N calves (13.6 ± 0.7 versus. 19.2 ± 0.8, respectively). During the willingness to approach tests B calves spent significantly ($P$<0.05) longer within 2 m of the unfamiliar person (14.7 ± 5.6 versus. 0.3 ± 0.0 seconds, respectively). Similarly, H calves spent significantly ($P$<0.01) longer within 2 m of the unfamiliar person (0.0 ± 0.0 versus 15.9 ± 5.9 seconds, for C or H calves respectively). Positive handling treatment significantly ($P$<0.001) reduced flight distance with C calves having a flight distance more than three times that of the H calves at 52 (± 0.5) days of age. This effect was still apparent ($P$<0.001) in H calves three weeks later (Figure 1).

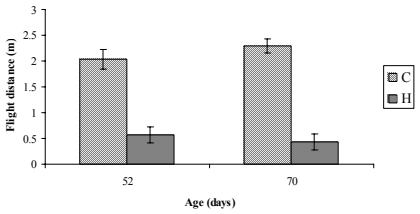


**Figure 1** Mean (± SEM) flight distance in the test arena at 52 (± 0.5) days of age ($P$<0.001) and 71 (± 0.4) days of age ($P$<0.001).

**Conclusion** Dairy calves remain calmer if they are blindfolded during disbudding, reducing the time taken to complete the procedure. Blindfolding may also prevent negative associations between humans and aversive procedures being made, but this requires further investigation. Positive handling of calves from birth until weaning was effective in reducing fear of humans, allowing the development of a good human-animal relationship. Taken together these results indicate that blindfolding during disbudding and positive human interactions may beneficial to both the calf and the stockperson.

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# The effect of grouping early lactation dairy heifers solely with heifers or with multiparous cows on eating, ruminating and resting behaviour

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**Introduction** Physiological challenges facing newly-calved heifers include parturition, adaptation to a concentrate-rich diet and a negative energy balance caused by the demands of lactation and continued growth. In addition, animals are subject to considerable psychological stress caused by new surroundings, social interactions with new herdmates and increased human contact. Abnormal behaviour, including prolonged time spent standing and reduced eating and rumination activity, may result from these combined challenges and contributes to further problems. The objective of this study was to assess the effect of housing early-lactation heifers solely with other heifers or in a mixed-parity group on the quantity and timing of eating, ruminating, lying, standing and stall use.

**Materials and methods** Twenty Holstein heifers (mean milk yield and bodyweight 20.1 (±0.5) kg/day and 569 (±12) kg respectively) were randomly assigned within 10 days of calving to one of two experimental groups: (i) loose-housed with early-lactation heifers (H-H), or (ii) loose-housed with early-lactation multiparous cows (H-C). All animals entered the appropriate group (mean total group size=59 animals) together and were identified by freeze-brands and coloured collars. Animals were offered a TMR *ad libitum*, once daily at 0700 h. Feed-barrier space (0.64 m/animal) and stall availability (1/animal) were the same in each group. Six management-imposed one-hour intervals were identified as peak feeding (0700-0800 h), post-peak feeding (0830-0930 h), quiet-time 1 (1100-1200 h), food push-up (1300-1400 h), return from milking (1500-1600 h) and quiet-time 2 (1600-1700 h), and assigned numbers 1 to 6 respectively. Each group was observed for 2 intervals per day. The intervals were randomised over 5 days, so that observations were made in each interval for each group at least once per 5-day week, and observations continued for 4 weeks. Within each interval, the 10 experimental animals in the appropriate group were observed for 30 seconds in turn, for a total of 10 times/h. At each observation the location of the animal (stall, passageway, or feed barrier), whether she was lying, standing or walking, and the activity engaged in (eating, ruminating or idling) was recorded. For each behaviour, mean observations per interval for each heifer week were used to calculate total weekly observations. A daily distribution score was calculated for each behaviour in every heifer week by multiplying the mean observations of that behaviour per interval by the interval number (1 to 6), so that the score for each activity increased the later in the day the behaviour occurred. The weekly total number of observations for each behaviour and the distribution scores were analysed by repeated measures ANOVA to assess the effect of group, week and group by week interactions (only reported if significant) on the total time engaged in specific behaviours and the daily distribution of behaviours.

**Results** All lying behaviour occurred within stalls. H-H heifers were observed ruminating, lying down or using stalls (lying and standing) more often than those in the H-C group, while H-C were observed eating more often than H-H (Table 1). Overall standing time did not differ between groups (Table 1) but there was a significant ($P$=0.022) group by week interaction (Figure 1). The daily distribution score did not differ between groups for any behaviour.

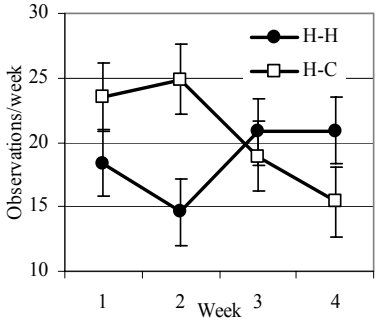


**Figure 1** Total observations (±SEM) of standing behaviour

**Table 1** Least squares means of total behaviour observations and behaviour distribution score

| | Group | | | *P* |
|---|---|---|---|---|
| Behaviour | H-H | H-C | SEM | Group |
| Total observations/week | | | | |
| Eating | 14.3 | 18.6 | 1.35 | 0.045 |
| Ruminating | 23.1 | 19.2 | 1.12 | 0.027 |
| Stall use | 38.7 | 31.5 | 1.80 | 0.013 |
| Lying | 25.5 | 19.4 | 1.77 | 0.031 |
| Standing (not at feed barrier) | 18.7 | 20.7 | 1.30 | 0.299 |
| Daily behaviour distribution score (higher = later in day) | | | | |
| Eating | 3.34 | 3.35 | 0.180 | 0.986 |
| Ruminating | 3.83 | 3.83 | 0.123 | 0.970 |
| Stall use | 3.59 | 3.53 | 0.092 | 0.669 |
| Lying | 3.54 | 3.09 | 0.157 | 0.065 |
| Standing (not at feed barrier) | 3.78 | 3.80 | 0.089 | 0.833 |

**Conclusion** Stall use (lying and standing) was less in H-C compared to H-H, despite equal provision of stalls in each group, suggesting that H-C heifers were subjected to intimidation by mature cows. Decreased stall use led to greater total standing time in H-C in the early weeks, but this declined as the study progressed, which probably coincided with establishment of stable social hierarchies within the groups. Conversely, there was no evidence of intimidation at the feed barrier in the H-C group, who were observed eating more often than the H-H group. With *ad libitum* provision of feed the H-C heifers may have learned to eat for longer by copying the mature cows. There was no significant shift in the distribution of specific behaviours throughout the day between groups, suggesting that the freedom of H-C heifers to express normal behaviour patterns was not restricted by the presence of mature cows. The results of this limited study suggest no detrimental effect of housing heifers and mature cows together, provided sufficient stalls and feed barrier space are available.

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**045**

## Subclinical mastitis changes the patterns of maternal - offspring behaviours in sheep

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**Introduction** Previous research has established the development and patterns of sucking behaviour in sheep, as well as factors potentially affecting them. However, in no case the health of the mammary gland of ewes, i.e. possible presence of mastitis, has been taken into account during these studies. In fact, not a single one of all relative research publications has considered examination of mammary secretion samples, to assess mammary health of the experimental ewes. The objective of the present work was to study whether ovine subclinical mastitis potentially influences dam/offspring behaviours during lactation, with a view to standardizing procedures during investigations of these behaviours.

**Materials and methods** In a study carried out under a licence for experimental procedures, 8 dairy Karagouniko-breed ewes each with a single lamb, were challenged on the 8th day of lactation (D0) by direct intramammary inoculation of approx. $5X10^6$ c.f.u. of *Staphylococcus simulans*; unilateral subclinical mastitis was induced (group I). Also, 4 ewes were included as uninoculated controls (group C). Standard bacteriological and cytological methods were used (Mavrogianni *et al.*, 2005). Ewe milk yield and lamb weights were measured. Behavioural data were collected by video cameras, using continuous focal observations on individual ewes and their lambs. Daily observation period was from 9 to 21 h daily, with an interval for feeding and grooming of ewes. Daily observation lasted 100 min for each ewe and was carried out in a pseudo-random manner to equally space data collection within the total observation period. Where proportions were compared, Chi-square test was employed. Two-sample t-test was used to compare paired data. For the remaining continuous data, we used analysis of co-variance with repeated measures taken as mean over time; the pre-challenge measurements were used as co-variate. The data were modelled by the general linear model applied in Minitab 14.

**Results** Inoculated mammary glands developed subclinical mastitis, confirmed by bacteriological and cytological evidence (Table 1). There was no significant difference in growth rate ($P$>0.08) among group I / group C lambs (daily growth: 227±17 g *vs* 220±24 g). Subclinical mastitis affected behaviours of ewes and lambs (Table 2). In group I, lamb behaviours were observed on 28:72 proportion between inoculated:control glands, whilst in group C, on 42:58 proportion among left:right glands, respectively; these differences first became evident on D3.

**Table 1** Cumulative results of milk parameters from ewes

| | Bacterial isolations | Positive CMT | Milk yield (ml) |
|---|---|---|---|
| Challenged glands (I) | 49/56†[a] | 51/56†[a] | 148±46[a] |
| Control glands (I) | 0/56[b] | 0/56[b] | 529±57[b] |
| All glands (C) | 0/56[b] | 0/56[b] | 593±105[b] |

† n/m = positive results out of total samples
a, b within the same column $P$<0.01
CMT = California Mastitis Test

**Table 2** Mean frequency of ewe (E) and lamb (L) behaviours observed

| | D-5 to D-1 | | D1 to D13 | | D14 to D26 | |
|---|---|---|---|---|---|---|
| Group | I | C | I | C | I | C |
| E-H† | 1.64 | 0.73[a] | 3.04 | 2.26[b] | 3.38[k] | 5.91[c,l] |
| E-HP† | 1.00 | 0.59[a] | 0.94 | 0.68[a] | 1.05[k] | 3.72[b,l] |
| E-V† | 1.58 | 1.08[a] | 0.81[k] | 0.38[b,l] | 0.38[k] | 0.20[b,l] |
| L-SA† | 11.38[a] | 13.72 | 10.93[a,k] | 14.34[l] | 8.73[b,k] | 12.13[l] |
| L-SS† | 9.44[a] | 12.18 | 9.64[a,k] | 12.37[l] | 7.33[b,k] | 9.74[l] |
| L-SB† | 4.13 | 4.57 | 3.13 | 4.02 | 3.71 | 4.97 |

† H: hindering sucking, HP: head-up posture, V: vocalization, SA: sucking attempt, SS: successful suck, SB: sucking bout
a, b, c within the same line (for same behaviour) $P$<0.05
k, l within the same stag $P$<0.05

**Conclusions** The findings indicate that subclinical mastitis affects dam and offspring lactation behaviour. Although no effects were seen in the growth of lambs, their sucking behaviour was clearly altered; lambs changed sucking pattern and were found to suck more frequently (up to 90%) the control gland of their dam. Obviously, lambs understood that the challenged gland could not fulfill their milk requirements. As there was no significant difference in frequency of sucking bouts among lambs of the two groups, one may postulate that ultimately lambs of both groups consumed similar quantities of milk, reflected in lack of growth differences. Thus, one wonders what might be the behavioural effects of subclinical mastitis in ewes rearing twins. Hence, there is a need for assessing the health status of the mammary gland in studies of lactational behaviour, in order to avoid conflicting results and standardise procedures in ethological research.

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**046**

# Methane output from beef cattle fed different high-concentrate diets

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**Introduction** Methane ($CH_4$) is a greenhouse gas of which the release into the atmosphere is directly linked with animal agriculture, particularly ruminant production. $CH_4$ emissions from ruminant also represent a loss in productive energy for the animal. Development of effective strategies to mitigate these methane emissions will have not only environmental benefits for the planet but also nutritional benefits for the animal. It has been shown that concentrate-rich diets result in a decrease in methanogenesis per unit of animal product (milk, meat). However, some of these diets may have adverse effects on the efficiency of production, e.g. due to risk of acidosis. Our study focused on measuring methane emissions on young bulls fed three contrasting finishing diets characteristics of three intensive levels of production in France.

**Materials and methods** Six Blond d'Aquitaine young bulls (average initial age and BW were 210 ± 30 days and 300 ± 22 kg, respectively) were randomly assigned to three dietary treatments in a 3 x 3 Latin square design. Each experimental period lasted 5 weeks, measurements occurring the last week of each period. Diets were 1) 63 % maize silage, 21 % maize grain and 16 % soya meal (MSM); 2) 49 % natural grassland hay, 41 % maize grain and 10 % soya meal (HM); 3) 14 % barley straw, 70 % maize grain, and 16 % soya meal (M). Animals had *ad libitum* access to diets distributed once daily. The desired forage:concentrate ratio was maintained by daily adjustment of offered amounts of forage and concentrate, depending on the composition of refusals of the previous day. Intake and total collection of feces were quantified daily on the last week of each period to determine total tract digestibility. At the same time, individual $CH_4$ productions were determined every day using the sulphur hexafluoride ($SF_6$) tracer technique as described by Pinares-Patiño et al. (2003). Incidence of acidosis was evaluated by measuring ruminal pH on fluid taken by rumenocentesis 5 hours after feeding on the last day of the period. All data were analyzed as a 3 x 3 Latin square using the MIXED procedure of SAS (1998). The statistical model included animal, period, diet. Fixed effects included period and treatment. Animal was the random effect. Overall differences between diet means were considered to be significant when $P < 0.05$.

**Results** The amount of OM ingested daily by bulls did not differ between diets (6.3 kg/d at mean; $P = 0.47$). Organic matter digestibility was significantly lower for HM diet than for the other two diets (- 4 % units at mean; $P < 0.01$). Mean daily $CH_4$ emission (L/d) was similar for bulls fed the diets MSM and HM but 56 % higher at mean than for bulls fed the diet M ($P < 0.001$). This difference between diets was maintained when $CH_4$ emissions were corrected for OM digested and GE intake. Approximately 7 % at mean of GE intake was lost as $CH_4$ for MSM and HM diets whereas only a 2.5 % loss of GE intake as $CH_4$ was observed for bulls fed the M diet. Estimated $CH_4$ production in L/kg liveweight gain was calculated from average daily gain measured in a separate experiment on 24 bulls fed the 3 diets (1.67 kg/d, 1.44 kg/d and 1.82 kg/d for MSM, HM, and M diets, respectively; Micol et al, unpublished data) and was 62 % lower for bulls fed the M diet than for the other two diets. As expected, the lowest ruminal pH was recorded for animals fed the M diet without clinical signs of acidosis. Bulls fed the HM diet tended ($P = 0.09$) to have lower ruminal pH than those fed the MSM diet.

**Table 1** Intake, digestibility, methane emissions and ruminal pH from bulls fed different high-concentrate diets

| | Diet | | | | |
|---|---|---|---|---|---|
| | MSM | HM | M | SE | Diet effect P-value |
| OM intake, kg/d | 6.42 | 6.44 | 6.05 | 0.245 | 0.47 |
| OM digestibility, % | 74.0[a] | 70.6[b] | 75.3[a] | 1.14 | < 0.01 |
| Methane | | | | | |
| L/d | 227.3[a] | 199.4[a] | 93.0[b] | 16.70 | < 0.001 |
| L/kg OM digested | 47.9[a] | 45.1[a] | 21.3[b] | 3.77 | < 0.001 |
| % GE intake | 7.2[a] | 6.6[a] | 2.5[b] | 0.45 | < 0.001 |
| Ruminal pH | 6.05[a] | 5.75[a] | 5.06[b] | 0.136 | < 0.001 |

[a,b]Means within rows with same superscript letters are not significantly different ($P > 0.05$).

**Conclusions** This study shows that the energy loss as methane was the lowest for the diet containing the highest quantity of concentrate without impairing animal intake or digestibility. The lower $CH_4$ losses from bulls fed the M diet might have been due to differences in ruminal fermentation as suggested by lower ruminal pH.

**Aknowledgments** to experimental cowshed workers and staff of the laboratory

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**047**

# The effect of absence of protozoa on methane emissions by lambs

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**Introduction** Livestock are one of the largest single sources of methane emission, equivalent to 15-20 % of total anthropogenic methane. Selective suppression of the rumen protozoa has been suggested to be promising approach to reduce methane release (Moss et al., 2000) as up to 25 % of the methanogens in the rumen are associated with protozoa (Newbold et al., 1995). However, contradictory results have been reported between *in vitro* and *in vivo* data and short and long term defaunation experiments (Ranilla et al., 2003). This study was carried out to investigate the effect of the absence of protozoa in the rumen on enteric methane production by lambs.

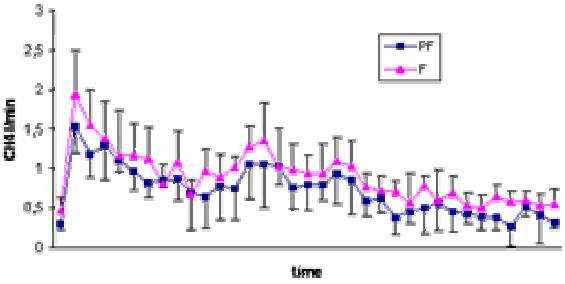
**Material and methods** Ten crossbred lambs were used. They were taken from the ewes within the first 24 h after birth and kept isolated from adult animals and bottle fed during 6 weeks. Once weaned (19.1 ± 3.23 kg) five of them were given orally 10 ml of rumen fluid collected from adult sheep rumen at the slaughter house (faunated group, F). The other five lambs were given 10 ml of rumen fluid that had been previously centrifuged at 1000 g for 10 min and then frozen and thawed to ensure the absence of viable ciliates and the presence of the same bacterial population given to F group (protozoa free group, PF). Thawed rumen fluid was examined by light microscopy to confirm the absence of viable protozoa due to the formation of crystals during freezing, which only affected protozoa. Both groups were kept isolated and fed a mix of concentrate supplement (Wynstay Lamb Finisher) and grass hay (60:40) during three months until they reached 40 kg of live weight. At 40 kg they were introduced in square polycarbonate chambers (1.8 x 1.8 m) to measure methane emissions over three days periods. Two chambers were used and in each period one lamb from each experimental group was housed in one chamber. Calculation of $CH_4$ emissions was based on the respective concentration measurements associated with airflows into and out of each chamber, which were obtained every 40 minutes. Animals were fed equal meals at 9:00 and 17:00 hours. Mean individual methane production was calculated for each lamb and statistically analysed by the following model; $Y_{ij}=\mu+P_i+E_{ij}$ where μ is the overall mean, P is the effect of absence of protozoa and E the error term using Genstat.

**Results** Protozoa-free lambs remained defaunated during the trial. No differences were observed on live weight gain and daily DM intake between PF and F lambs. Protozoa free lambs had lower ($P<0.05$) daily methane emissions and methane produced per kg of dry mater intake than faunated lambs (Table 1). Overall PF lambs produced 26 % less methane than F lambs. The hourly methane produced (Figure 1) showed an immediate post feeding rise in the morning followed by a decline and a second rise after the 17:00 h feeding and then declined slowly to basal levels.

**Table 1** Intakes, live weight and methane emissions

| | PF | F | SEM | *P* |
|---|---|---|---|---|
| Live weight. kg | 49.9 | 48.6 | 2.67 | 0.906 |
| DM intake. g/day | 1198 | 1209 | 69.2 | 0.913 |
| $CH_4$ l/day | 26.0 | 35.2 | 2.82 | 0.049 |
| $CH_4$ l/kg LW | 0.52 | 0.71 | 0.044 | 0.024 |
| $CH_4$ l/kg DM intake | 21.6 | 29.0 | 1.41 | 0.006 |

**Figure 1** Hourly pattern of methane emissions




**Conclusions** Our results indicate that the absence of protozoa in the rumen of lambs decreased $CH_4$ emissions by 26 % and show variability in methane produced over the day as affected by the feeding pattern. More research is needed to further investigate the effect of the absence of protozoa on methane production under different feeding regimes and diets.

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**048**

# Effect of forage type on methane production from dairy cows

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**Introduction** Methane ($CH_4$) has attracted considerable attention in recent years because of its contribution to the greenhouse effect, as it has a 100-year global warming potential 23 times that of carbon dioxide (IPCC, 2001). Dairy cows are major contributors to the production of methane from the agricultural sector in Europe because of their large numbers and high emission factors. Methane production is affected by several factors, one of which is diet; for example, the type of forage offered to ruminants has an important effect on methane production (Boadi *et al.,* 2004). The aim of this experiment was to investigate how different forage types affected methane production from dairy cows.

**Materials and methods** Six mid-lactation Holstein cows were used in this experiment. Three forages were offered *ad libitum*: grass silage; maize silage; and fermented whole crop wheat. Each forage was supplemented with 5kg concentrates per cow per day. The metabolisable energy content of the diets was approximately 12 MJ/kgDM. The cattle were offered each diet in pairs in a complete changeover study (3 periods). After a diet adjustment period of approximately 2 weeks, each pair of cows underwent a three-day energy balance period and animals were transferred to calorimetric chambers for three days, with methane production being measured for the final 48 hours. An analysis of variance (ANOVA) was carried out on the data, which was blocked for treatment, using the Genstat statistical package.

**Results** Results presented in Table 1 show the effect of the three treatments on animal and dietary parameters. Forage type had a significant (P<0.001) effect on forage and total dry matter intake (DMI) in both the balance and the chamber periods. DMI, as a proportion of metabolic liveweight, was significantly (P=0.002) affected by the treatments. There was no significant treatment effect on milk yield during the balance and chamber periods. There was a significant (P<0.001) difference in methane production between different forages. Feeding of maize silage resulted in the greatest methane production per day, followed by whole crop wheat, with grass silage producing least methane. There was also a significant (P<0.001) difference in methane production per kilogram liveweight between the three different forages. There was a significant (P<0.05) difference in methane production as a proportion of DMI between the three treatments, with grass silage having the highest methane emission per kg DMI. There were no significant treatment effects on methane production as a proportion of milk yield.

**Table 1** Effect of forage type on production and methane emissions*

| | | Grass silage | Maize silage | Whole crop wheat | s.e.d. | sig. |
|---|---|---|---|---|---|---|
| Balance period | Liveweight (kg) | 537.7 | 550.9 | 547.5 | 13.81 | NS |
| | Forage DMI (kg/d) | 9.84[a] | 13.70[b] | 14.17[b] | 0.626 | *** |
| | Total DMI (kg/d) | 13.88[a] | 17.71[b] | 17.92[b] | 0.713 | *** |
| | Milk Yield (kg/d) | 12.9 | 15.5 | 15.9 | 1.34 | NS |
| Chamber period | Forage DMI (kg/d) | 8.99[a] | 13.63[b] | 13.64[b] | 0.880 | *** |
| | Total DMI (kg/d) | 12.82[a] | 17.61[b] | 17.38[ab] | 1.013 | *** |
| | Milk Yield (kg/d) | 14.3 | 15.7 | 14.7 | 0.98 | NS |
| | $DMI/LW^{0.75}$ ($kg/kg^{0.75}$) | 0.115[a] | 0.155[b] | 0.154[b] | 0.0089 | ** |
| | Methane (l/d) | 485[a] | 609[b] | 544[ab] | 16.3 | *** |
| | Methane/LW (l/kg) | 0.90[a] | 1.11[b] | 0.99[ab] | 0.04 | *** |
| | Methane/MY (l/kg) | 34.5 | 42.1 | 38.8 | 3.30 | NS |
| | Methane/DMI (l/kg) | 38.3[a] | 34.7[ab] | 31.4[b] | 2.39 | * |

* DMI = DM intake; LW = liveweight; MY = milk yield
sig. = significance; *** = p<0.001, ** = p<0.01, * = p<0.005, NS = not significant

**Conclusions** Methane emission per kg DMI was higher with cows offered grass silage than those given maize silage or whole crop wheat, although this was largely due to a lower DMI with grass silage.

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**049**

# Nutrition index and soil nitrate residues in grazed pastures fertilised with mineral fertiliser, pig slurry or cattle compost

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**Introduction** A code of good practice was established by each European member state according to the nitrate directive. In Belgium, the nitrogen (N) inputs in pastures from slurry or compost are limited to 210 kg N/ha. Larger quantities can be applied when additional measurements, including soil nitrates analyses are carried on by the farmer. This trial aims to measure nitrogen balance, nitrogen nutrition index (NNI) and soil nitrates contents in pastures fertilised with mineral nitrogen fertiliser, pig slurry or cattle compost, the pastures being grazed by dairy cows and the fertilisation being brought at similar efficient N levels. NNI was calculated as the ratio of the actual N concentration to the sward N concentration it would have to be at a similar biomass in order to sustain a non limiting growth and a biomass accumulation (Lemaire and Gastal, 1997). Cattle compost was produced from cattle manure unloaded through the beaters of a spreader.

**Materials and methods** A total of 40 - 45 cows grazed in a rotational system 3 paddocks allocated to the same treatment during 5 years. The first paddock was fertilized with cattle compost (C), the second with pig slurry (S) and the third with mineral nitrogen fertiliser (Min N). The grass chemical composition was determined before each grazing. Botanical composition was determined each year. The grass heights were measured before and after the grazing. The cows were milked twice per day and milk productions were recorded at each milking. Milk from tank was sampled at each milking in order to measure milk urea. The animals were weighed before and after each paddock grazing. Soil cores were taken up to 60 cm depth (0-30 and 30-60 cm) in November and March in order to determine the soil nitrate content. Nitrogen surplus was calculated by the difference between N inputs and N outputs. N inputs were composed by the legume fixation (Farrugia et al, 1997), atmospheric N (35 kg/ha) and N from fertilisation. N efficiency was estimated at 30 % in C (back-effects included), at 50 % in S and at 100% in min N. N in milk, concentrate and live weight gains represented N outputs. Linear models including years and treatments effects were used for statistical analyses

**Results** N inputs by fertilisation were different at 169, 170 and 102 kg N/ha in C, S and Min N plots respectively. N inputs as efficient N were 51 kg in C and 85 kg in S. The grass yield was significantly higher in S than in C ($p<0.05$), the grass yield in Min N being intermediary (table 1). There were no differences in grazing heights and NNI owing to similar grazing managements in the 3 plots. The grazing duration expressed as herd grazing days, the milk yields and milk urea contents were not significantly different. The N surplus was significantly lower in Min N than in S and C ($P<0.05$). The apparent N efficiency, calculated as the ratio of the N output (milk and live weight gain N) to the N input (N from atmospheric deposition, fertilizer, concentrate, fixation by leguminous), was significantly lower in S and C than in Min N ($P<0.05$). Nitrate residues in soil were generally lower than 35 kgN-NO3/ha (Figure 1). They were significantly higher in the 0-30 cm depth than in the 30-60 cm depth (29.7 vs 12.7 kg N-NO3/ha; $P<0.001$). There were no significant differences between fertilisation systems (19.8, 19.7 and 29.0 kg N-NO3 in C, S and Min N) for the nitrate contents in soil.

**Table 1** Grass characteristics and N balance

| | *Compost* | *Slurry* | *Min N* | *SEM* |
|---|---|---|---|---|
| Grass | | | | |
| yield (kg DMha$^{-1}$) | 10016a | 11052b | 10736ab | 218 |
| height in (cm) | 13.3a | 13.3a | 12.9a | 0.3 |
| height out (cm) | 5.1a | 4.9a | 5.1a | 0.15 |
| NNI (%) | 72.3a | 66.9a | 76.5a | 3.7 |
| Grazing days (d ha$^{-1}$) | 11.6a | 11.04a | 11.0a | 0.2 |
| Milk yields (kg ha$^{-1}$) | 7718 | 7435 | 7144 | 145 |
| Milk urea (mg/l) | 290a | 321a | 333a | 17.5 |
| N balance (kg ha$^{-1}$) | | | | |
| -N legume fixation | 38.4a | 35.2a | 30.6a | 7.8 |
| -N total input | 255a | 251a | 179b | 12.4 |
| -N milk | 43.2a | 42.0a | 40.0a | 1.0 |
| -N surplus | 208a | 204a | 134b | 12.2 |
| **-app N efficiency** | 19.8a | 19.6a | 26.2b | 2.0 |

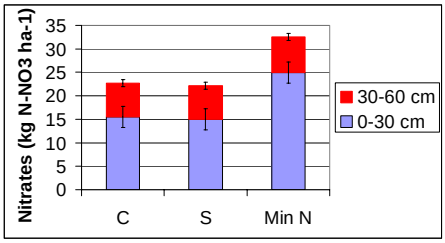


**Figure 1** Soil nitrate content in grazed pastures fertilised with cattle compost, pig slurry or mineral nitrogen fertiliser. (C: compost, S: slurry, Min N: mineral nitrogen fertiliser)

**Conclusion** The use of pig slurry and cattle compost as compared with mineral N fertiliser increased N balance and reduced apparent N efficiency. The grazing days and the milk yields per ha were not different. The nitrate contents in soil were not increased by use of slurry or compost. The low nitrate contents suggested a low nitrate leaching with the three types of fertilisation.

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**050**

# Genetic progress in broiler traits – implications for welfare

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**Introduction** Genetic selection has dramatically increased the growth potential of broilers. A positive correlation exists between growth rate and the occurrence of leg disorders (Sørensen *et al*., 1999), and rapid growth rate is widely presumed to be a fundamental cause of leg ill-health in the modern broiler. However, it is recognised that the simultaneous improvement of growth rate and reduction in incidence of leg disorders is possible (Sørensen, 1992). The objective of this study was to explore the impact that selection for growth has had on broiler welfare, as measured by leg health and mortality, using data from genetic control strains.

**Materials and methods** Birds from three Modern lines (female-MFL, male-MML and an intermediate male-MIML line; subjected to balanced selection for conventional broiler characteristics and welfare traits) and their corresponding 1972 Control lines (discrete populations, randomly selected to maintain the characteristics of the 1972 commercial broiler; CFL, CML, CIML) were fed standard broiler diets (Ross Broiler Management Manual, 2002). Birds were reared in a windowless, temperature controlled house in 60 pens, each with 30 birds at day-old. Stocking density was maintained below 34kg/$m^2$ at all times. Bulk weighings were made at 0, 10, 21, 28, 35 and 42 days (for all lines) and also at 56, 70 and 77 days (Control lines). Six birds per pen were removed for processing at 28, 35 and 42 days (Modern lines) and at 42, 56 70 and 77 days (Control lines). Processing performance was then corrected by interpolation to a common weight of 2kg. Mortality was recorded daily to cause. Leg health, in males only, was assessed on all birds in two pens per treatment at a weight of approximately 2kg (37 and 70 days for the Modern and Control strains respectively). Birds were categorised as having good leg health (no defects), twisted toes, bows or some other defect (evidence of tibial deformity, TD or leg twists). Data were analysed in Minitab using a General Linear Model.

**Results** The Modern lines were significantly heavier (P=0.000), had a significantly better feed conversion ratio (FCR) at 2kg (P=0.000) and a significantly higher breast meat yield at 2kg (P=0.000) than the Control lines. There was no significant difference in mortality at any age between Modern and Control lines (Figure 1). The number of birds considered to have good leg health was generally better in the Modern lines than in the Control lines despite a significant difference in growth rate. The birds not showing good leg health were mainly categorised as showing evidence of TD or leg twists (19.8% for the Modern lines vs. 10.8% for the Control lines), the incidence of bows was greater in the Control lines than in the Modern lines (19.3% compared to 3.3% respectively), while the incidence of twisted toes was low in both lines (1.0% for Modern lines and 2.1% for Control lines).

**Table 1** Liveweight (LWT), feed conversion ratio corrected for mortality (FCR), breast meat yield (BM), mortality and % birds with good leg health (GLH) in Modern (2005) and Control lines (1972). Mean of males and females,

| Line | | 42d LWT (g) | 2kg FCR | 42d mort. (%) | 2kg BM g/kg | 2kg GLH (%) |
|---|---|---|---|---|---|---|
| Modern | MML | 2880 | 1.57 | 5.0 | 186 | 73.3 |
| | MFL | 2449 | 1.72 | 3.3 | 161 | 66.7 |
| | MIML | 2455 | 1.71 | 4.3 | 174 | 87.5 |
| Control | CML | 1204 | 2.26 | 5.3 | 112 | 82.8 |
| | CFL | 1216 | 2.19 | 4.7 | 109 | 54.8 |
| | CIML | 1229 | 2.24 | 1.8 | 118 | 65.6 |
| *SEM* | | *51.0* | *0.031* | *1.30* | *2.10* | *8.37* |

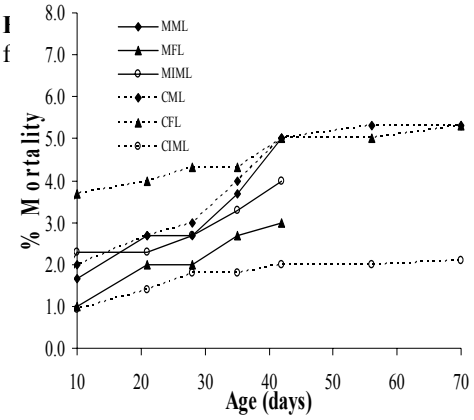


**Figure 1** Changes in mortality in Modern and Control lines

**Conclusions** The differences in performance between the Modern and Control lines were significant. Despite these differences in performance there was no significant difference in mortality between the Modern and Control lines, whilst leg health was generally better in the Modern lines. The Modern lines in this study were selected using a Best Linear Unbiased prediction (BLUP) of breeding values for a range of traits including growth rate and leg health. The data from this study show that it possible to select for increased growth rate with no detriment to welfare as measured by leg health and mortality.

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**051**

# Farmer satisfaction: potential for genetic evaluation in Ireland?

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**Introduction** Previous studies have indicated genetic variation in traits describing the overall satisfaction of a farmer with daughters of a particular sire (Cue *et al.,* 1996; Visscher and Goddard, 1995). Such traits are useful in determining cow-level factors contributing to overall farmer satisfaction as well as providing supplementary information in sire selection. The objective of this study was to evaluate whether genetic variation in a subjectively scored trait reflecting farmer satisfaction existed in Ireland and to attempt to elucidate the factors contributing to farmer satisfaction.

**Materials and methods** In 2005 and 2006, farmers participating in the national dairy sire progeny testing scheme were asked to score all their first lactation animals for overall satisfaction using a scale of 1 to 5: 1=strongly disliked, 2=disliked, 3=average, 4=liked, and 5=liked very much. Following the removal of herds that reported no variation in animal satisfaction, scores on 2,396 primiparous animals across 316 herds remained. An animal model including the fixed effects of herd-year, age at first calving and days in milk at scoring was fitted in ASREML (Gilmour *et al.,* 2004); the dependent variable was the exponent of farmer satisfaction score. A series of bi-variate analyses were also undertaken to estimate genetic and residual covariances between farmer satisfaction and total lactation yield for milk, fat and protein as well as fat and protein composition and somatic cell score (i.e., $\log_e$ somatic cell count). Pedigree information three generations deep was collated; the pedigree file consisted 5,982 non-founder animals. Spearman rank correlations between farmer satisfaction and the individual animal's economic breeding index (i.e., the total merit index in Ireland) and type traits were also undertaken; a total of 1,119 animals had information on farmer satisfaction, EBI and type traits.

**Results** The heritability for farmer satisfaction was 0.13 (SE=0.051) indicating that 13% of the phenotypic variation in satisfaction of cows across 316 Irish herds was due to additive genetic effects. The heritability of 0.13 corroborates previous estimates on similar traits in New Zealand (0.12; Cue *et al.,* 1996) and Australia (0.18 to 0.23; Visscher and Goddard, 1995). Genetic correlations with milk production were similar in sign although stronger than the corresponding phenotypic correlations with higher milk production associated with more favoured animals. Interestingly, the correlations with milk composition were negative suggesting that farmers were more satisfied with animals that had lower milk composition. This is at odds with expectations and attempts will be made in the future to try and explain this phenomenon. One potential reason could be the association between milk composition and some trait of importance to Irish farmers that is currently not measured or analysed. Although the standard errors were relatively large, there was a general tendency for farmers to be more satisfied with animals of better udder health. A positive, although weak, correlation ($r=0.07$; $P<0.05$) existed between farmer satisfaction and economic breeding index indicating that farmers were more satisfied with animals scoring high on the Irish dairy cattle total merit index. Furthermore, a stronger positive correlation ($r=0.27$; $P<0.001$) between farmer satisfaction and overall type was evident indicating that conformation was also an important characteristic of a cow in the eyes of the farmer. Farmers tended to favour taller, wider, deeper, more angular animals with silky, well supported and attached udders and that walked with an even gait.

**Table 1** Phenotypic and genetic correlations (standard errors in parenthesis underneath) between farmer satisfaction and milk production and composition.

| Trait | Milk yield | Fat yield | Fat percent | Protein yield | Protein percent | SCS |
|---|---|---|---|---|---|---|
| Phenotypic | 0.33 (0.020) | 0.20 (0.022) | -0.15 (0.023) | 0.31 (0.021) | -0.12 (0.023) | -0.04 (0.023) |
| Genetic | 0.55 (0.204) | 0.26 (0.314) | -0.28 (0.216) | 0.40 (0.224) | -0.25 (0.206) | -0.06 (0.289) |

**Conclusions** Results from this study suggest some similarity across farmers in factors affecting their overall satisfaction, as indicated by the existence of a heritability of 0.13. It is therefore feasible to produce estimated breeding values for sires for overall farmer satisfaction. Farmers tended to be more satisfied with cows of superior economic breeding index and overall type. Early predictors can be derived in a multi-trait analysis including traits such as milk production that are genetically correlated with farmer satisfaction. It may also be possible to derive conversion equations for farmer satisfaction in Ireland based on proofs for milk production and/or type in other countries.

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**052**

# Developing a robustness index for UK dairy cows

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**Introduction** Selection for increased milk production may have resulted in the "shift" in nutrient partitioning towards milk production away from maintenance of functional fitness. This has led to the conclusion that dairy cows appear to be less 'robust' than in the past. The current national index, £PLI, combines the predicted transmitting ability (PTA) for production and functional traits, multiplied by their relative economic values, with the overall goal of maximising cow profit. Stott et al. (2005) updated economic weights for £PLI, increasing the relative weight on functional traits, but showed that the genetic responses in functional traits were still expected to decline, albeit at a slower rate compared to selection on production alone. The aim of this work is to develop a Robustness Index for UK dairy cattle that halts the predicted genetic decline in health and fertility traits using a restricted index approach.

**Materials and methods** The initial index upon which the restricted indices were developed was current £PLI (below) which has profit as the goal of the index using combinations of milk, fat and protein kg., lifespan, mastitis, lameness and fertility as goal traits.
£PLI = (Milk PTA× -0.04) + (Fat PTA× 1.10) + (Protein PTA× 2.90) + (Lifespan PTA× 32.00) + (Somatic cell count PTA× -0.20) + (Locomotion PTA× 1.30) + (Calving interval PTA× -0.31) + (Non-return rate PTA× 1.56)
Annual responses to selection on this index were calculated based on a national progeny test scheme with 75 daughters per bull. Restricted indices were developed using selection index methodology and the correlated responses calculated. £PLI, and its economic, genetic and phenotypic parameters, was used as a starting point (see Stott et al., 2005 for more detail). The selection objective was changed to restrict the response in the trait(s) of interest to zero while still maximising the rate of genetic improvement in the overall selection objective. Health and fertility traits were restricted to zero change/cow/year one by one (calving interval, mastitis, lameness and non-return rate) to examine the impact on individual trait and overall economic response.

**Results** Table 1 shows that selection on current £PLI, which has included fertility since August 2006, resulted in an expected annual response of £11.53 per cow per annum. This economic response is due to genetic improvement in production traits and overall lifespan. However, the goal of maximising profit allows for a continued decline in functional traits. Table 1 shows the impact of restricting health and fertility traits compared to selection on current £PLI. The predicted total economic response is expected to decline as more health and fertility traits are restricted to zero change. There is a 10.8% (£1.25 per cow per year) decline in economic response when all health and fertility traits in the goal are restricted to 'no change' relative to selection on £PLI where these traits are unrestricted. The results show that halting the decline in functional traits reduces profit per cow compared to a profit based index such as £PLI as the improvements in functional traits cannot offset the benefit of genetic improvement in production on overall profit.

**Table 1** Expected annual responses to selection for production, lifespan, health and fertility traits[1] when selecting profit based and restricted indices.

| Index | Total resp. (£) | Expected annual genetic responses | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Milk (kg) | Fat (kg) | Protein (kg) | LS ($lact^{ns}$) | MAST (cases) | LAME (cases) | CI (days) | NR (0/1) | CS (0-9) |
| £PLI | 11.53 | 49.29 | 3.59 | 3.00 | 0.045 | 0.0018 | 0.0015 | 0.337 | -0.004 | -0.027 |
| Restricted | | | | | | | | | | |
| Non-return | 11.11 | 37.40 | 3.13 | 2.71 | 0.052 | 0.0007 | 0.0008 | 0.19 | 0 | -0.028 |
| +Calving Int | 10.95 | 27.63 | 2.84 | 2.48 | 0.063 | 0.0002 | 0.0009 | 0 | 0 | -0.023 |
| +Mastitis | 10.95 | 27.02 | 2.84 | 2.46 | 0.063 | 0 | 0.0008 | 0 | 0 | -0.023 |
| +Lameness | 10.28 | 21.80 | 2.59 | 2.14 | 0.071 | 0 | 0 | 0 | 0 | -0.024 |

[1] LS = Lifespan, MAST= Mastitis, LAME = Lameness, CI = Calving interval, NR = Non-return rate, CS = Condition score

**Conclusions** The predicted genetic decline in health and fertility traits can be constrained, however there is a "knock-on" effect on production traits and therefore a decrease in the predicted overall economic response per cow per year using current economic assumptions. This drop in profitability per cow per year can be considered the cost to farmers to start to reverse the decline in health and fertility traits and/or the societal cost of such welfare improvements. Assuming a national dairy herd of 2 million cows, the adoption of an index halting the genetic decline in health and fertility would have a cumulative cost of £2.5 million per annum.

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**053**

# Characterization of dairy farm environments and their effect on cow lifespan

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**Introduction** Environmental sensitivity exists when an environmental difference has more effect on some genotypes than on others (Falconer and Mackay, 1996). Quantifying the degree of environmental sensitivity shown by individual sires allows us to identify sires as specialists (those which rank highly in certain environments) or generalists (those which rank similarly across environments). Dairy farm environments vary enormously and this affects the production and health of the cows. The aims of this study were (1) to relate detailed survey information on farm management systems to information available at the national level to provide a definition of farm environment, and (2) to assess the effect of environment on lifespan.

**Materials and methods** Firstly we performed a canonical correlation analysis of survey and national data. This involved calculation of maximal correlations between linear combinations of survey (F) and national (G) measurements. The national variables in this analysis comprised herd solutions for milk, fat and protein yields, mean age at 1st calving, herd size, temperature (temp) and rainfall (rain). The survey variables, specific to the farms in our survey, and chosen to reflect different farming systems, were months not housed (out), number of cows (cows), regular veterinary visits (vet; 0 = no, 1 = yes) and amount of concentrates fed (tonnes/cow/year). The analysis was based on measurements from 419 herds.
The lifespan of a cow can be considered an overall indicator of the health of the animal in a specific environment. To assess the effect of environment, lifespan data (Brotherstone et al., 1997) were extracted for daughters of 1000 widespread sires. The total number of daughters with data was ~ 400 000. We fitted a random regression model to the data with a random intercept and slope for each sire:

$$Y_{ij} = \text{constant} + \text{sire}_i + \text{herd}_j + (\text{sire x herd})_{ij} + \bar{s}\, G_j + (s_i - \bar{s})\, G_j,$$

where $Y_{ij}$ is the lifespan score for daughters of sire i in herd j, $G_j$ is the environment score for herd j , and $s_i$ is the 'sensitivity' for the $i^{th}$ sire. The herd and (sire x herd) terms represent residual effects (herd or sire x herd effects unexplained by overall regression on $G_j$ or variation in the slope of that regression). The environmental score $G_i$ is a linear combination of national variables produced by the canonical correlation analysis (see below).

**Results** The largest canonical correlation was 0.62. The first canonical variables, scaled in each case so that the maximum coefficient was +1, were:
(G) Temp + 0.296*Age – 0.175*Protein + 0.092*Rain + 0.090*Fat − 0.005*Milk
(F) Concentrates + 0.553*Vet + 0.332*Cows – 0.005*Out
The first canonical F variate was interpreted as a measure of system input, large values corresponding to high input farms (high concentrate usage, frequent veterinary visits, large number of cows which spend a lower than average time out at grass). We suggest the corresponding G variate as a measure of system input for general use.

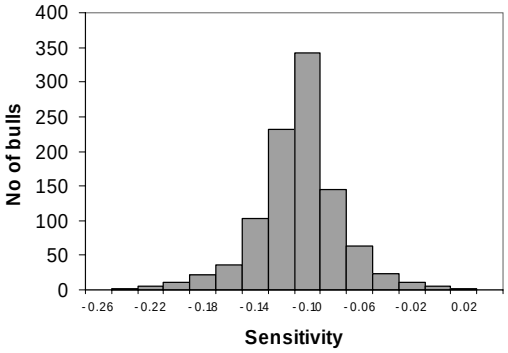


**Figure 1** Histogram of sensitivity (slope) BLUPs

Lifespan variance components for intercept and slope were statistically significant. Figure 1 shows a histogram of sensitivity BLUPs for the 1000 sires represented in the data set. For most sires the sensitivity coefficient is negative, indicating that lifespan tends to be greater in low-input environments.
The effect varies from sire to sire. Daughters of sires with sensitivity coefficients of around zero tend to be long-lived in any environment (i.e. generalists) whereas daughters of sires with negative coefficients tend to live longer in a low-input than a high-input environment (i.e. specialists).

**Conclusions** The intensity of production and the climate were the most important factors in differentiating between farms. Nationally recorded variables can be used as environmental descriptors. Significant environmental sensitivity for lifespan was shown, indicating that sires produce daughters that are sensitive to the environment to differing degrees.

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**054**

# Comparison of metabolic profiles and fertility in the same dairy cows during their first and second lactations

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**Introduction** The decline in dairy cow fertility over the past 30 years has major economic, welfare, genetic and environmental consequences. A significant number of potential replacement heifers either never calve or else complete only a single lactation. The relationships between metabolic status and fertility may change with age as cows reach physical maturity. To determine the reasons behind the high loss rates attributable to infertility, this study tracked a group of animals until the end of their second lactation to compare fertility and metabolic hormone profiles in the same cows at different ages.

**Materials and methods** A cohort of consecutively born Holstein-Friesian heifer calves (n=134) from the same herd were recruited at birth. At first calving as primiparous cows (PP) and second calving as multiparous cows (MP), blood samples were collected at -1 week prepartum and at 1, 2, 4, 6, 8 and 12 weeks postpartum for measurement of IGF-I, insulin and glucose. Weight and body condition score (BCS) measurements were made at the same times. Cows were housed throughout early lactation and fed a total mixed ration. Services were by AI at observed oestrus. Fertility parameters were monitored from milk progesterone (P4) profiles and herd records and recorded as days to commencement of luteal activity (CLA), calving to conception interval and services per conception. Data were analysed using repeated measure ANOVA and Pearson correlations.

**Results** Of the 134 calves recruited, 12 (9%) died or were culled before service, 4 aborted and were culled and 10 animals did not conceive or aborted in their first service period and were then re-inseminated, so calved for the first time as 3 year olds. No further information on these 26 animals is given. Data were therefore obtained from 108 PP cows calving as 2 year olds and of these 94 went on to calve again.

**Table 1** Summary of milk production and fertility parameters in the first 2 lactations

| | PP cows | MP cows | P |
|---|---|---|---|
| No. calved | 108 | 94 | |
| No. culled during that lactation | 14 (13%) | 26 (28%, to be confirmed) | |
| Peak milk yield (kg/day) | 32 ± 0.6 | 38 ± 0.6 | P<0.001 |
| Total milk yield (kg) | 8925 ± 200 | 10154 ± 218 | P<0.001 |
| Days to CLA | 19.2 ± 1.7 | 26.2 ± 1.7 | P<0.001 |
| Length 1st luteal phase (days) | 19.5 ± 0.7 | 14.6 ± 1.0 | P<0.001 |
| Normal P4 profiles | 66/102 (65%) | 26/84 (31%) | P<0.001 |
| No. services/conception | 2.3 ± 0.17 (4 not served) | 2.2 ± 0.16 (14 not served) | NS |
| Calving-to-conception interval (days) | 143 ± 10 (n = 99) | 139 ± 10 (n = 67). | NS |

Milk production increased with age as expected. Following their first calving, PP cows had significantly shorter days to CLA and a longer first luteal phase than following the second calving but these parameters were not correlated in individuals (P>0.1). A greater proportion of PP cows had normal progesterone profiles and the progesterone profile in the first lactation was not predictive of that in the second (P=0.3). Overall fertility parameters were similar between lactations.
Metabolic data were also compared between the first and second lactations. IGF-I concentrations were significantly higher throughout the peripartum period in PP cows (P<0.001). In MP cows insulin concentrations were similar pre-calving, but decreased more in weeks 1-2 postpartum, followed by recovery to a higher concentration, so there was a significant age x time interaction (P<0.001). Glucose concentrations remained similar throughout the peripartum period in both age groups. MP cows were heavier throughout (P<0.001) and lost more weight in the postpartum period (P<0.02). BCS measurements were lower throughout in the PP cows (P<0.001), but BCS loss was similar between age groups. In PP cows the interval to CLA was negatively related to IGF-I in the pre-calving and service periods (P<0.01), whereas in the MP cows it was negatively related to BCS in the service period (P<0.05). In the PP cows the calving-to-conception interval was negatively related to pre-service BCS and BCS loss (P<0.05) and in the MP cows it was negatively related to weight loss (P<0.05). PP cows which either took >200 days to conceive again (n=16) or failed to conceive at all in the first lactation (n=5) calved with lower IGF-I concentrations and BCS (P<0.05) in comparison with more fertile cows.

**Conclusions** These results show that only 50% of heifers born alive remained in the herd after 2 lactations. Poor fertility in the first lactation in this study was associated with poor BCS (<2) and lower IGF-I at calving, although we have previously shown that excess BCS (>3, Wathes *et al*., 2007) also causes fertility problems in PP cows. Good heifer management prior to calving is therefore critical to reduce subsequent losses due to infertility.

**Acknowledgements** The support of Defra and the Milk Development Council is gratefully acknowledged.

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**055**

# Effect of body condition score at calving and diet energy content post calving on the fertility of dairy cows during early and mid-lactation.

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**Introduction** Poor reproductive performance is a major problem when considering the sustainability of the modern high yielding dairy cow, with reproductive costs being estimated at £500 million per annum across UK dairy herds (Lamming *et al.,* 1998). Armstrong *et al.* (2001) stated that poor nutrition causes delayed puberty, aberrant oestrous cycles, lowered conception rates and reduced calf birth weight. Excessive and prolonged negative energy balance (NEB) has, on numerous occasions, been suggested as a major contributor to poor fertility and subsequent increased culling rates. In the present study the effects of a range of dietary treatments on energy balance and reproductive performance were evaluated.

**Material and methods** Forty Holstein heifers and forty Holstein cows between 2$^{nd}$ and 6$^{th}$ parity were allocated to one of four treatments, based on a 2x2 factorial design: high or low energy density diet pre- and post calving. All diets were offered as a TMR. 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.* (A) and restricted (R) diets (6 kg DM/ day) depending on their respective treatments. The pre-calving treatment for cows commenced 100 days prior to the 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 (R) to 6 kg DM complete diet/ day, while the high energy pre-calving diet continued to be fed *ad lib.* (A). Post calving, treatments were balanced for parity, body weight and date of calving. The concentrate: forage DM ratios of the high (H) and low (L) energy density diets post calving were 70:30 and 28:72 respectively, providing 12.5 and 11.7 MJ ME/ kg DM. Consequently, there were four treatment groups; AH, AL, RH and RL. All post-partum animals were housed as a single group. Progesterone concentration in milk was measured twice weekly on Tuesday and Friday (AM milk samples). Calving commenced on 1 September and breeding commenced on 30 November. Animals were inseminated at a minimum of 42 days post calving, on the first observed standing heat. All fertility events were recorded until six months after the commencement of breeding. Data were analysed using multiple linear regression. The average daily energy balance (ADEB) for each cow was calculated for each week of lactation, using 'The Feed into Milk' equations (Thomas 2004).

**Results** Post calving dietary treatment had a significant effect on the average daily energy balance (ADEB) ($P \leq 0.001$). The range in ADEB for individual cows/heifers in the first 21 days of lactation was +33 to -114 MJ/d. Conception was defined by pregnancy verification (Pd positive) at 100d post artificial insemination. Overall, 86% of cows intended for breeding conceived during the six month breeding period. Pre- or post calving treatment had no significant effect on any of the reproductive parameters. The average pregnancy rate to first service was low (25%), with the average number of services per conception being 2.8. The average 100d in calf rate from the beginning of the breeding period was 58%. The average peak concentration of milk progesterone was significantly ($P=0.005$) affected by parity (negative relationship) and showed a significant pre/post treatment interaction ($P=0.05$). Only the variables listed in Table 1 had a significant relationship with fertility parameters.

**Table 1** The relationship between selected variables (X) and reproductive parameters (Y) using the model "Y = Constant + Parity + X".

| Y* | X | Probability | Regression Relationship |
|---|---|---|---|
| Weeks to CLA | Weeks to energy nadir | 0.017 | Positive |
| Average LP | ADEB (1-21 days) | 0.017 | Positive |
| Average LP | ADEB (1-42 days) | 0.015 | Positive |

* CLA=Commencement of luteal activity (days from calving), LP= luteal phase duration (days), (n=80).

**Conclusions**. The results indicate that post calving dietary regime had a major effect on energy balance but a limited effect on reproductive performance. On an individual cow basis there were significant influences of cow energy status on parameters relating to ovarian function, indicating adverse effects of severe NEB.

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**056**

# The effect of energy balance and condition score on early lactation fertility in two genetic lines of Holstein cattle maintained in two contrasting feeding systems

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**Introduction** The ability of high-yielding modern dairy cows to perform in the range of systems found on UK dairy farms, and to be profitable and sustainable, needs investigating. As part of a project to study the robustness of such animals, milk-progesterone profiling was used to investigate the reproductive performance of two genotypes of dairy cow on two production systems (Pollott and Coffey, 2006). In this paper differences in fertility between genotypes and systems was investigated using energy balance (EB) and body condition score (CS) as explanatory factors.

**Material and methods** SAC's Crichton Royal 200 cow dairy herd is split between two genetic lines; the Selection Line comprised daughters of bulls selected for the highest fat and protein production whilst the Control Line comprised daughters of bulls selected for having average genetic merit at the time of insemination. Each line was further subdivided and half fed on a high concentrate diet and half on a high forage diet, cows being randomly allocated to a feeding system at first calving and remaining in that group until disposal. Cows were milked thrice daily and condition scored weekly. These measurements were used to estimate daily energy content (EC) of the cows, which in turn were used to estimate the daily change in energy content (or energy balance; EB), the nadir of EB (EBN), the day on which it occurred (DEBN), the mean change in energy per day during the first 25 days of lactation (AEB) and the day on which the cow returned to positive energy balance (EBP). Equivalent estimates for condition score were also calculated; daily change in (CSC), nadir of CSC (CSCN), the day on which it occurred (DCSCN), the mean change in CS during the first 25 days of lactation (AVCSC25) and the day of return to positive CSC (CSP). The milk progesterone profile data (PRG), described by Pollott and Coffey (2006), was used to define the commencement of luteal activity (CLA) and cows were routinely recorded for heats and services. Day of first heat (HEAT1) and day of first service (SERVE1) were also calculated. REML methodology was used to investigate the relationship between the three measures of fertility (CLA, HEAT1 and SERVE1) and the energy balance/condition score traits. Fixed effects of genetic line (GEN), system (SYS) and lactation number (LNO) were used in the statistical models as appropriate. Cow was fitted as a random effect. Skewed traits were log transformed and least-squares means were estimated from untransformed data.

**Results** Data were available for 330 lactations from 214 cows, recorded between September 2003 and January 2006. A summary of the significant effects influencing the three fertility traits is shown in Table 1 for energy balance traits and Table 2 for condition score traits.

**Table 1** Summary of significant effects influencing the three early lactation fertility traits.

| | Energy balance models | | | | | | | Condition score models | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | GEN | CLA | HEAT1 | PRG | EC | AEB | DEBN | CLA | HEAT1 | PRG | CS | ACS | CSP |
| CLA (d) | * | | | * + | *** - | ** - | NS | | | ** + | *** - | ** - | NS |
| HEAT1 (d) | NS | *** + | | NS | * - | NS | *** - | *** + | | NS | NS | NS | NS |
| SERVE1 (d) | NS | NS | *** + | ** - | NS | ** - | NS | NS | *** + | * - | NS | NS | * + |

* P<0.05, ** P<0.01, *** P<0.001; + and – show the direction of slope of the effect

The effect of system on CLA reported by Pollott and Coffey (2006) disappeared when both EB and CS effects were used in the model. CLA (EB analysis) was the only trait affected by genetic line; the least-squares means for control and selection line cows were 30.1 and 36.7d respectively. Earlier CLA was associated with higher levels of both CS and EC, lower progesterone levels at first cycle, and less average daily loss in both CS and EB up to the 25$^{th}$ day of lactation. Earlier day of first heat was highly influenced by earlier CLA, higher EC and a higher nadir of EB drop. Day of first service was decreased by an earlier day of first heat, higher progesterone levels at first cycle, and higher average EB loss during the first 25 days of lactation. Day of first service was also associated with an early return to positive condition score.

**Conclusion** The recent decline in dairy cattle fertility has been linked to higher production and the increased use of Holstein genes. In this study most of the effects of higher performance studied were linked to feeding level and mediated through either energy balance or condition score changes in the cows. However, a genetic effect on CLA was found which could not be explained by any other recorded factor in the study, indicating a change in the genetic makeup of the selected group not found in the control cows.

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# Effects of dairy cow genotype and plane of nutrition on nitrogen partitioning between milk and body tissue

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**Introduction** Breeding programmes for the Holstein-Friesian have historically focused on improved milk production with little emphasis on functional traits such as fertility or disease resistance. In contrast, Norwegian dairy cattle have been bred using a multi-trait selection procedure. Differences in selection procedures for the two breeds may have major effects on the efficiency of food use and partitioning of nutrients that may offset the potential advantages of improvements in functional traits. Recently, Yan *et al.* (2006) reported that Holstein-Friesian cows partitioned more energy into milk and less to body tissue than Norwegian cows. The objectives of the present study were to examine possible differences in nitrogen partitioning and the efficiency of N utilization between Holstein-Friesian and Norwegian dairy cows.

**Materials and methods** Sixteen first lactation dairy cows (8 Holstein Friesian and 8 Norwegian) were used in a 2 (breed) * 2 (plane of nutrition) factorial design study. Each breed was offered two levels of concentrates (proportion of total diet) for days 1-100 (0.60 v. 0.30), 101-200 (0.50 v. 0.20) and 201-308 (0.40 v. 0.10) of lactation. The concentrates consisted of 0.230, 0.225, 0.300 and 0.245 kg/kg fresh weight of barley, wheat, sugar beet pulp and soyabean meal respectively. The remainder of the diet was primary growth grass silage. Total diet nutrient digestibility studies were undertaken on each animal at day 80, 160 and 240 of lactation. During each measurement period, cows were tied in individual standings in a cowshed for 8 days with feed intake and faeces and urine output being measured during the final 6 days. Data were analysed by ANOVA to examine effects of breed and plane of nutrition using experiment period as block.

**Results** The mean N partitioning averaged over 3 periods are presented in Table 1. There was no significant interaction between cow genotype and plane of nutrition on any variable. In comparison with the low plane of nutrition, the high plane significantly reduced faecal N ($P<0.001$), urine N ($P<0.05$) and manure N ($P<0.001$) excretion as a proportion of N intake, and significantly increased retained N as a proportion of N intake ($P<0.05$), irrespective of cow genotypes. Milk N/N intake was similar between the planes of nutrition.

**Table 1** Effects of cow genotype and plane of nutrition on nitrogen utilisation

| | Holstein | | Norwegian | | | Significance | |
|---|---|---|---|---|---|---|---|
| Plane of nutrition | High | Low | High | Low | s.e. | Breed | Plane | BxP |
| Faecal N/N intake | 0.29$^{a}$ | 0.37$^{c}$ | 0.29$^{a}$ | 0.34$^{b}$ | 0.008 | | *** | |
| Urine N/N intake | 0.35$^{ab}$ | 0.33$^{a}$ | 0.37$^{b}$ | 0.34$^{a}$ | 0.010 | | * | |
| Manure N/N intake | 0.64$^{a}$ | 0.70$^{c}$ | 0.66$^{ab}$ | 0.68$^{bc}$ | 0.012 | | *** | |
| Milk N/N intake | 0.26 | 0.25 | 0.24 | 0.24 | 0.009 | | | |
| Retained N/N intake | 0.10$^{b}$ | 0.05$^{a}$ | 0.10$^{b}$ | 0.08$^{ab}$ | 0.017 | | * | |

Cow genotype had no significant effect on the efficiency of N utilisation, although Holstein-Friesian cows produced a slightly higher milk N/N intake with both planes of nutrition, and a marginally lower retained N/N intake with the low plane of nutrition. However, when examining cow genotype effect in individual periods (Figure 1), Holstein-Friesian cows partitioned more N into milk and less into body tissue in early lactation, although this effect was not observed in mid lactation. In late lactation there was no difference in milk N/N intake between genotypes but Holstein-Friesian cows partitioned more N into body tissue than Norwegian cows. These results agreed with breed effects on energy partitioning reported by Yan *et al.* (2006).

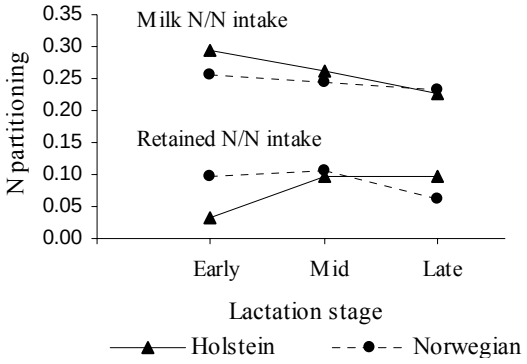


**Figure 1** Effect of cow genotype on N utilisation

**Conclusions** Holstein-Friesian cows partitioned more consumed N into milk and less into body tissue than Norwegian cows in early lactation. However in late lactation, Holstein-Friesian cows deposited more N into body tissue.

**Reference** Yan, T., Mayne, C. S., Keady, T. W. J. and Agnew, R. E. 2006. Effects of dairy cow genotype (Holstein-Friesian versus Norwegian) with two planes of nutrition on energy partitioning between milk and body tissue. *Journal of Dairy Science* **89:** 1031-1042.

**058**

# Prepubertal predictors for fertility in dairy cattle: potential use of metabolic hormones

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**Introduction** The length and severity of negative energy balance postpartum is unfavourably correlated (genetically & phenotypically) with interval to first ovulation (de Vries & Veerkamp, 2000). During this period concentrations of free fatty acids (FFA), glucose, growth hormone (GH), insulin, insulin like growth factor 1 (IGF-1) and other hormones, all of which have links with many aspects of reproduction, are altered. A moderate heritability and genetic correlation between these parameters in the female and male (who is ultimately of most use for selection purposes) calf with female fertility could allow juvenile selection for fertility. With this in mind the objective of this study was to estimate the heritability of FFA, glucose, GH, insulin and IGF-1 concentration in male and female calves.

**Materials and Methods** Plasma samples were collected from Holstein Friesian calves (126 ± 12.7 d old; mean±s.e.) during two studies A (n=496 females; 1996-2001; 7 commercial dairy herds) and B (B1, n=326 females, B2, n=256 males; 2002-2006; MOET breeding scheme). Glucose and FFA were determined by commercial assay kits (Glucosehexokinase II, Bayer, USA; NEFA C, Wako Chemicals GmbH, Germany respectively). Insulin, GH and IGF-1 were determined by time-resolved fluoro-immunometric assays (Løvendahl *et al.*, 2003; Løvendahl & Purup, 2002; Frystyk *et al.*, 1995). Data for FFA, GH, Insulin and IGF-1 were log-e transformed to produce normally distributed residuals. Univariate mixed linear models were fitted using ASREML software (Release 2.0 VSN International. Ltd., UK). The fixed effects were sire percentage Holstein, sex, age, experimental batch (accounting for farm and sampling day) and sex.batch interaction. Random terms were fitted for additional residual variance in subset A, B1 and B2 where necessary. The genetic relationships were modelled by the relationship matrix calculated from the three-generation pedigree.

**Results** Heritabilities of FFA, glucose, GH, insulin and IGF-1 were moderate and significant (*Table 1*). Heritability estimates varied within trait between the three datasets (A, B1 & B2) due to differences in the error variance, whilst the genetic variance remains comparable within trait. The differences in the error variance may be due to inconsistencies in experimental protocol particularly in dataset B1. Animals in B1 were often given minimal time to acclimatise to the experimental environment prior to sampling, possibly increasing the effects of "stress". The effect of sex, day and sex.day interaction was significant in all the analyses whereas the effect of age was only significant for insulin and IGF-1.

**Table 1** Animal numbers, mean concentration, error variance ($\sigma^2_E$) and heritability ($h^2$) for FFA (μeqv/L), glucose (mmol/l), GH (ng/ml), insulin (pmol/l) and IGF-1 (ng/ml) in each dataset

| Trait | N | Mean (±s.e) | $\sigma^2_E$ | $h^2$ (±s.e) |
|---|---|---|---|---|
| FFA-A | 490 | 261.47 (5.55) | 0.051 | 0.25 (0.13)* |
| FFA-B1 | 301 | 128.32 (5.45) | 0.162 | 0.09 (0.05)* |
| FFA-B2 | 238 | 266.24 (10.30) | 0.127 | 0.12 (0.06)* |
| Glucose-A | 490 | 4.27 (0.04) | 0.223 | 0.23 (0.11)* |
| Glucose-B1 | 301 | 4.73 (0.05) | 0.454 | 0.13 (0.06)* |
| Glucose-B2 | 238 | 5.07 (0.05) | 0.261 | 0.20 (0.10)* |
| GH-A | 495 | 2.70 (0.10) | 0.398 | 0.15 (0.07)* |
| GH-B1 | 301 | 3.65 (0.17) | 0.309 | 0.18 (0.09)* |
| GH-B2 | 238 | 6.29 (0.34) | 0.484 | 0.13 (0.06)* |
| Insulin-A | 495 | 30.20 (1.43) | 0.328 | 0.10 (0.06)* |
| Insulin-B1 | 301 | 49.64 (2.03) | 0.266 | 0.12 (0.06)* |
| Insulin-B2 | 238 | 39.14 (2.01) | 0.127 | 0.22 (0.12)* |
| IGF-1-A | 486 | 160.73 (3.41) | 0.070 | 0.55 (0.13)* |
| IGF-1-B1 | 298 | 125.24 (4.61) | 0.308 | 0.21 (0.05)* |
| IGF-1-B2 | 236 | 265.52 (7.35) | 0.040 | 0.66 (0.14)* |

P<0.05=*; P<0.01=**; P<0.001=***

**Conclusion** Although the heritability estimates in this study varied between the three datasets, the additive genetic variance remained consistent and significantly different from zero. This research has indicated that an appropriate level of genetic variation is present in all traits investigated to potentially be useful for juvenile selection criterion provided this is following a strict experimental procedure for some hours before sampling and management practices within the population of animals used is stringent to avoid additional error variance. The next stage of this work will be to investigate the genetic, phenotypic and environmental (co) variation between these traits and fertility and to investigate the correlated responses, to indirect selection, in other traits such as production.

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# Effect of dietary polyunsaturated fatty acids on uterine endometrial gene expression of enzymes involved in prostaglandin biosynthesis in cattle

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**Introduction** The modern high-producing dairy cow is sub-fertile. Early embryo loss, within the first 16 days of gestation, is the greatest contributor to reproductive wastage (Sreenan *et al*., 2001), though the causes for this are not well understood. Nutrition plays a fundamental role in reproduction and specifically there is emerging evidence that supplemental polyunsaturated dietary fatty acids (PUFA) can increase cow fertility (Thatcher *et al*., 2006). For example, *in-vitro* studies suggest that the ω-3 PUFAs, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) may be pivotal in the suppression of endometrial prostaglandin $F_{2\alpha}$ synthesis, a critical regulator of luteolysis and hence embryo survival, though the biochemical pathways involved have not been elucidated. The objective of this study was to determine the effect of dietary supplementation of ω-3 PUFA on mRNA expression of key uterine endometrial genes involved in $PGF_{2\alpha}$ biosynthesis.

**Materials and methods** Reproductively normal crossbred beef heifers (n=7 per treatment) were individually fed a straw and barley/beet pulp based concentrate which was supplemented with a rumen protected source of either saturated fatty acid (palmitic acid;Low PUFA) or EPA/DHA (High PUFA 273g DM) per head per day for 45 days. Both diets were isolipid and isonitrogenous. Following slaughter, uterine endometrial tissue from both groups was harvested and stored at -80°C. Analysis of fatty acids in endometrial tissue was carried out using gas chromatography. Total RNA was isolated from fragmented frozen endometrial tissue using TRIzol reagent. RNA quantity was determined spectrophotometrically. RNA quality was assessed using the Agilent Bioanalyzer 2100. One microgram of each sample of RNA was reverse transcribed to generate cDNA. Primers were designed to amplify specific fragments of the following 10 genes known to be involved in the prostaglandin biosynthetic pathway, phospholipase C (PLC), phospholipase $A_2$ ($PLA_2$), cyclooxygenase I and II (COX I and II), prostaglandin E synthase (PGES), prostaglandin F synthase (PGFS), 15-hydroxy prostaglandin dehydrogenase (15-HPD) and peroxisome proliferator activated receptors (PPAR) α, δ, and γ. SYBR Green quantitative real time RT-PCR reactions were performed to measure the relative expression of these genes. Ubiquitin was used as a reference gene following evaluation of a number of 'housekeeping' genes using the 'GeneNorm' software package. All amplified PCR products were sequenced to verify their identity. Gene expression results were calculated using the $2^{-\Delta\Delta CT}$ method. Significance of differences between the groups was tested using PROC GLM, SAS.

**Results** Combined EPA and DHA endometrial concentrations were more than two-fold higher in the high compared to the low group ($P<0.05$), while EPA concentrations alone were more than four-fold higher in the high group ($P<0.01$). Furthermore, concentrations of arachidonic acid, the main substrate for $PGF_{2\alpha}$ synthesis, were lower in tissues from the high group (0.296 mg $g^{-1}$ versus 0.188 mg $g^{-1}$) ($P<0.001$). Expression of mRNA for prostaglandin E synthase (PGES) and the peroxisome proliferator-activated receptors, PPAR α and δ was increased in animals fed the high compared with low PUFA diet ($P < 0.05$) in response to long chain ω-3 PUFA supplementation (Figure 1). Mean phospholipase $A_2$ ($PLA_2$) gene expression tended to decrease by 2.1 fold in animals fed the high PUFA diet, ($P=0.08$). The other 6 candidate genes were not differentially expressed in animals fed the high PUFA diet.

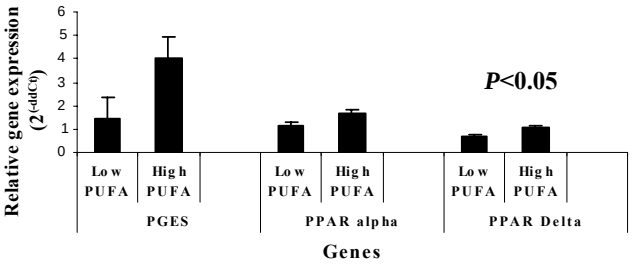


**Figure 1** Real time RT-PCR analysis of endometrial genes involved in prostaglandin synthesis

**Conclusions** Genes controlling $PGE_2$ biosynthesis and PPAR production were shown to be significantly up-regulated by PUFA supplementation. $PGE_2$ in uterine fluid has previously been reported to be associated with enhanced embryo development and survival rates. There is further evidence to suggest that PPAR-δ is involved in the pregnancy recognition process of cattle and that it mediates at least some of the beneficial effects of long chain ω-3 PUFA supplementation (MacLaren *et al*., 2006). Results from this study therefore suggest a possible mechanism by which PUFAs may influence uterine function and in turn embryo survival in cattle. The data may contribute to the development of nutritional management strategies to increase cow fertility.

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**060**

# Body condition score and reproductive performance in dairy cows in Greece

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**Introduction** Various studies in dairy cows have shown that their Body Condition Score (BCS) effectively reflects their nutritional status and energy content (Ferguson *et al*. 1994). Extremes in BCS and BCS losses have been identified as a risk factor for health problems and reduced reproductive efficiency (Markusfeld *et al*. 1997, Lopez-Gatius *et al*. 2003). Body condition scoring is easy and inexpensive to perform and gives a reliable estimate of body energy reserves. Hence, in practice, BCS can be used as a tool for ration formulation and management decisions concerning reproduction of dairy cows (Banos *et al*. 2004). The objective of this study was to determine and quantify the impact of BCS in the reproductive performance of primiparous Holstein cows.

**Materials and methods** The experiment was conducted in a large commercial dairy farm (900 cows) located in Northern Greece. All heifers calving between January 2005 and June 2006 were included in the study. A trained veterinarian assessed their body condition at calving and thereafter every week during the first three months of lactation. A five-point scale (1=emaciated, 5=obese, scored in 0.25 point intervals) and the method described by Ferguson *et al*. (1994) were used. Repeated BCS records from 299 animals were obtained. Based on their BCS at calving, cows were assigned into two groups: those with BCS ≤ 2.50 (L1) and those with BCS ≥ 2.75 (H1). Based on the minimum BCS observed during the first three months of lactation, cows were also classified in two further groups: those with a minimum BCS ≤ 2.25 (L2) and those with a minimum BCS ≥ 2.50 (H2). Reproductive performance data included conception rate at first service, interval (days) from calving to first service, interval from calving to conception and number of services per conception. Data were analysed by ANOVA using BCS level as factor.

**Results** The average BCS at calving was 2.73 (SD: 0.483; n=299), whereas mean BCS for groups L1 and H1 at calving were 2.30 (SD 0.213; n=144) and 3.12 (SD 0.296; n=155), respectively. The average minimum BCS during the first three months of lactation was 2.16 (SD: 0.351; n=299), whereas mean minimum BCS for groups L2 and H2 were 2.00 (SD 0.221; n=229) and 2.65 (SD 0.222; n=70), respectively. Data on reproductive performance of these cows at first artificial insemination (AI) are shown in Table 1. Reproductive performance of cows confirmed pregnant by October 2006 (n=153) after the first and subsequent AI are shown in Table 2. The interval to conception in relation to the average number of services in L1 group was likely the result of missed heats and early embryonic deaths.

**Table 1** Reproductive performance of primiparous cows according to BCS level at calving (L1= low, H1=high) and minimum BCS level (L2= low, H2= high )

| | L1 (n=144) | H1 (n=155) | Significance | L2 (n=229) | H2 (n=70) | Significance |
|---|---|---|---|---|---|---|
| Conception rate at 1st AI | 14.6% | 23.9% | * | 15.7% | 31.4% | ** |
| Days from calving to 1st AI | 96 | 90 | NS | 95 | 85 | NS |

* P<0.05; ** P<0.01

**Table 2** Reproductive performance of primiparous cows confirmed pregnant by October 2006 according to BCS level at calving (L1= low, H1= high) and minimum BCS level (L2 = low, H2= high )

| | L1 (n=85) | H1 (n=68) | Significance | L2 (n=120) | H2 (n=33) | Significance |
|---|---|---|---|---|---|---|
| Days from calving to conception | 218 | 147 | *** | 205 | 118 | *** |
| Services per conception | 2.5 | 1.9 | ** | 2.4 | 1.6 | ** |

** P<0.01; *** P<0.001

**Conclusion** Results to-date extend previous findings on the relationship of BCS during early lactation with the reproductive performance of primiparous cows. Low BCS at calving (≤2.50) and low minimum BCS (<2.25) during the first trimester of lactation have a statistically significant adverse effect on conception rate at 1st service, mean interval from calving to conception and average number of services per conception. Body condition scoring should be an integral part of dairy herd health and reproduction management.

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**061**

# Genetic correlations between the cross country, dressage and showjumping phases of eventing competition

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**Introduction** Competition data is widely used in the genetic evaluation of racing (Belhajyahia *et al.,* 2003) and showjumping horses (Reilly *et al.,* 1998). However, its use in the evaluation of eventing horses is rare. In a previous study, we investigated the use of competition results for the evaluation of eventing horses by examining the component parts of eventing competition – dressage, showjumping and cross country (Kearsley, unpublished). The aim of this study is to estimate genetic correlations between the different phases of eventing competition at pre novice and novice level as part of a project to predict breeding values for British sport horses.

**Materials and methods** The data consisted of penalty points for the cross country, dressage and showjumping phases of eventing competition at pre novice and novice level. Pre novice is the lowest level of eventing competition and novice is the next level up.

**Table 1** Datasets for pre novice and novice level

| | Total Records | No. Horses | No. Sires |
|---|---|---|---|
| Pre Novice | 116532 | 6296 | 595 |
| Novice | 106486 | 3890 | 504 |

The penalty points for each phase were converted to normal scores with a method adapted by Royston (1982). The traits to be analysed were the penalty points for cross country, dressage and showjumping at pre novice and novice level. The data was analysed with a trivariate sire model using ASReml (Gilmour *et al.,* 2002).

$$y_{ghijklm} = \mu_g + \alpha_{gh} + \beta_{gi} + \gamma_{gj} + u_{gk} + v_{gkl} + w_{gm} + e_{ghijklm}$$

Where $y_{ghijklm}$ is the trait value for horse *l,* with sire *k*, of sex *h* and age *i*, competing at phase *g* in competition *j* with rider *m.* $\mu_g$ is the overall mean, $\alpha_{gh}$ is the effect of sex, $\beta_{gi}$ is the effect of age, $\gamma_{gj}$ is the effect of competition, $u_{gk}$ is the effect of sire, $v_{gkl}$ is the effect of the individual horse, $w_{gm}$ is the effect of the rider and $e_{ghijklm}$ is the residual error. The effects of sex, age and competition are fixed; the effects of sire, horse and rider are random. $u_{gk}$, $v_{gl}$, $w_{gkm}$ and $e_{ghijklm}$ were normally distributed with variance/covariance matrices of the form $\mathbf{\Sigma_u \otimes I}$, $\mathbf{\Sigma_v \otimes I}$, $\mathbf{\Sigma_w \otimes I}$ and $\mathbf{\Sigma_e \otimes I}$, respectively.

**Results** Moderate genetic correlations of a similar range were estimated between all three phases at pre novice level (0.36-0.46). More variation is observed in the genetic correlations at novice level, with a fairly high correlation between cross country and showjumping (0.58), a moderate correlation between cross country and dressage (0.35) and a low correlation between dressage and showjumping (0.13). However, the SE's are large so the differences in the correlations between grades may be due to sampling error. The heritabilities predicted here are similar to those predicted in the previous within phase study.

**Table 2** Genetic correlations and heritabilities (diagonal) for cross country, dressage and showjumping

| | | Cross Country | Dressage | Showjumping |
|---|---|---|---|---|
| Pre Novice | Cross Country | 0.03±0.010 | | |
| | Dressage | 0.46±0.174 | 0.08±0.016 | |
| | Showjumping | 0.44±0.154 | 0.36±0.120 | 0.08±0.014 |
| Novice | Cross Country | 0.04±0.014 | | |
| | Dressage | 0.35±0.209 | 0.07±0.019 | |
| | Showjumping | 0.58±0.150 | 0.13±0.161 | 0.09±0.018 |

**Conclusions** The genetic correlations presented here could be subject to error due to the high SE's, however, they do make structural sense. A high correlation is observed between cross country and showjumping, which is to be expected because they both demand jumping ability. The lower correlation between showjumping and dressage was not wholly unexpected as studies into the correlations between ability in specialised dressage and showjumping competitions have shown little to no genetic correlation (Huizinga and van der Meij, 1989). Most of the genetic correlations presented here are of sufficient magnitude to predict ability in one phase from another, and given the low heritability of cross country in comparison to the other two phases, these between phase correlations could prove important in the future prediction of breeding values for eventing horses.

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**062**

# The genetic parameters of lactation using a biological model on cows from UK dairy herds

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**Introduction** Recently, Pollott (2000, 2004) has proposed a biological model of lactation which mimics the three processes of cell proliferation, apoptosis and milk secretion rate per cell during lactation. This model has been used on dairy sheep and cattle to investigate several aspects of its utility to account for the factors which influence the milk production during lactation. Pollott and Gootwine (2001) and Pollott (2002) reported genetic parameters of the biological model for a single flock/herd sheep respectively. Here we report an extensive genetic study of over 400 UK dairy herds using the biological model of lactation.

**Material and methods** The dataset used in this research was provided by the National Milk Records Ltd. from commercial dairy herds in the UK. The lactations were recorded between 1994 and 2004, and came from the largest 431 herds in the dataset. The lactation records used in this analysis were edited as follows: lactations that had the first test day record (TD) recorded at >80$^{th}$d of lactation and lactations with less than 4 TD were deleted. Subsequently, cows without pedigree information were discarded. Furthermore, heifers <20 or >40 mo of age at first calving (AFC) were deleted. After editing, there were 161,799 animals in the dataset with 353,502 lactations from which 82,255 were first lactation records. The number of TD per lactation ranged from 4 to 15, with an average of 10. The two-parameter multiplicative model (Pollott, 2000) was used to analyse the lactation records and the curve characteristics described by Pollott (2000) were calculated. The traits used in the genetic analysis were maximum secretion potential (kg; MS), relative cell death rate (DR), daily increase in milk production mid-way between calving and peak yield (kg; GM), peak yield (kg; PY), day of peak yield (DP), persistency (i.e., rate of decline in milk yield midway between the peak and the end of lactation in kg; DM), total milk yield (kg; TMY) and calculated total milk yield (kg; CTMY). Heritabilities and correlations reported here were for first lactation records only and were estimated with an animal model in the ASREML software package using a 3-generation pedigree, single-generation pedigree and fixed effects and covariates as appropriate.

**Results** The pedigree file used for these analyses contained 216,869 animals. The trait means and SD for the 8 traits analysed are shown in Table 1. The heritabilities, genetic and phenotypic correlations for the traits are also shown in Table 1.

**Table 1** Means, SD, heritabilities (bold), genetic (above the diagonal) and phenotypic (below the diagonal) correlations of heifer lactation curve traits.

| | Mean | S.D. | MS | GM | DP | PY | DR | DM | CTMY | TMY |
|---|---|---|---|---|---|---|---|---|---|---|
| MS (kg) | 30.1 | 5.1 | **0.28** | 0.85 | 0.24 | 0.99 | -0.08 | 0.58 | 0.95 | 0.95 |
| GM (g/d) | 151.2 | 44.3 | 0.74 | **0.12** | -0.24 | 0.83 | 0.64 | 0.88 | 0.66 | 0.63 |
| DP (d) | 34.6 | 7.0 | -0.07 | -0.56 | **0.03** | 0.27 | -0.69 | -0.42 | 0.45 | 0.51 |
| PY (kg) | 28.7 | 5.0 | 1.00 | 0.69 | -0.04 | **0.30** | -0.12 | 0.54 | 0.96 | 0.96 |
| DR | 0.001 | 0.0004 | 0.25 | 0.84 | -0.39 | 0.17 | **0.08** | 0.84 | -0.69 | -0.40 |
| DM (g/d) | 47.7 | 22.0 | 0.57 | 0.76 | -0.10 | 0.51 | 0.88 | **0.08** | 0.31 | 0.31 |
| CTMY (kg) | 7,688 | 1,321.0 | 0.83 | 0.34 | 0.13 | 0.92 | -0.51 | 0.14 | **0.32** | 0.99 |
| TMY (kg) | 7,609 | 1,310.3 | 0.78 | 0.30 | 0.14 | 0.82 | -0.21 | 0.09 | 0.99 | **0.34** |

Standard error of heritabilities < 0.01; s.e. of correlations range from 0.002 to 0.05.

The level of genetic variation in total milk yield is in the range commonly reported for dairy animals. The maximum secretion potential of the lactation and the peak yield have similar heritabilities and correlations with other traits. This analysis confirms the observation by Pollott and Gootwine (2001) that MS and PY are almost identical traits. Both MS and PY are both strongly correlated with total milk yield. Most other traits had low heritabilities but there were some high correlations between them; notably GM with most other traits; and DR with DM and the total milk yield traits.

**Conclusion** Some components of the biological model of lactation have a moderate level of genetic variation and have high genetic correlations with total milk yield traits. The use of these genetic parameters in an appropriate selection index could contribute towards changing the shape of the lactation curve. This may be particularly important if early-lactation characteristics are detrimental to profitability and sustainability and need to be improved whilst still maintaining overall milk yield.

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# Inbreeding and inbreeding depression in Irish Holstein-Friesian cattle

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**Introduction** Inbreeding occurs when related individuals are mated to each other. Inbreeding reduces milk production, and impairs health, fertility and survival; a phenomenon known as inbreeding depression. Smith *et al*. (1998) reported losses in milk yield of 27 kg per 1% increase in inbreeding in US Holsteins. The objective of this study was to investigate the level of inbreeding in Irish Holstein-Friesian cattle and to quantify its effect on milk, fat and protein production and somatic cell count.

**Materials and methods** Pedigree information of animals born after 1950 was extracted from the Irish Cattle Breeding Federation database. Holstein-Friesian animals were defined as all crosses between Holstein and Friesian animals and were selected for analysis (n=2,851,081). The number of complete generation equivalents (CGE) with pedigree information was computed and was used as a measure of pedigree completeness. Generation intervals were calculated along each selection pathway (i.e., sires of males, dams of males, sires of females and dams of females) and an average generation interval was calculated. Inbreeding coefficients were calculated using the Meuwissen and Luo (1992) algorithm. Total lactation yield of milk, fat and protein as well as lactation average somatic cell score (SCS; $\log_e$SCC) were available on up to 138,577 lactations from 89,987 cows with full pedigree information on at least 3 generations and calving in either 2003, 2004 or 2005. Fixed effects included in the sire repeatability model run in ASREML (Gilmour *et al*., 2004) were herd-year-season of calving, parity, age at calving nested within parity and lactation length. Inbreeding was treated as a continuous variable and in a separate series of analyses as a class variable with 5 levels (F=0, 0<F≤6.25, 6.25<F≤12.5, 12.5<F≤25, and F>25). Non-linear effects of inbreeding and interactions between inbreeding and parity were also tested for significance in the model. A pedigree file 3 generations deep was also created, containing 17,696 non-founder animals.

**Results** The level of inbreeding increased at a rate of 0.1% per annum in the Holstein-Friesian population from 1995 to 2005 (P<0.001) to the current level of 1.48%. Of the calves born in 2005, 59% were inbred. The average generation interval across all four pathways was 6.3 years. Thus, the average rate of increase in inbreeding per generation was 0.63%. Pedigree completeness increased by 0.8 CGE per annum (P<0.001) between 1985 and 2005, reaching 4.9 CGEs in 2005. The average inbreeding of animals used in the depression analysis was 2.43%. Table 1 shows the results from the regression analyses undertaken. Inbreeding had a non-linear effect on milk production, manifesting itself as a negative impact only at inbreeding levels greater than 4%. A significant interaction existed between parity and inbreeding for protein yield, fat percentage and SCS. Protein yield decreased by -0.10, -0.27, -0.40, -0.42, and -0.36 (all SE≤0.13), while SCS increased by 0.002, 0.008, 0.010, 0.012 and 0.016 (all SE≤0.004) for parities 1 to 5 respectively, and fat percentage decreased by -0.004, -0.002, -0.002, -0.0002 for parities 1 to 4, respectively, and increased by 0.0002 in parity 5 animals (all SE≤0.0016). Effect of inbreeding depression on milk, fat and protein are lower than those reported previously (Smith *et al*., 1998), as inbreeding is relative to production; average 305-day yield for animals in the present study was 7,118kg compared to the 8,794kg mature equivalent for US Holsteins reported by Smith *et al*. (1998).

**Table 1** Model solutions (SE included in parenthesis) for the effect of inbreeding on milk yield and composition

| | Milk(kg) | Fat(kg) | Fat(%*$10^{-3}$)† | Protein(kg)† | Protein(%*$10^{-3}$) | SCS(*$10^{-2}$)† |
|---|---|---|---|---|---|---|
| Linear | 0.03 (2.56) | -0.4(0.06) | - | - | -1.6(0.52) | - |
| Quadratic | -0.4(0.15) | - | - | - | 0.1(0.03) | - |
| 0<F≤6.25 | 6.8(16.74) | 1.9(0.67) | 24.3(7.39) | 0.2(0.51) | 2.0(3.44) | 7.4(1.41) |
| 6.25<F≤12.5 | -47.5(21.98) | -0.9(0.88) | 15.3(9.66) | -1.9(0.68) | -0.1(4.50) | 10.7(1.86) |
| 12.5<F≤25 | -160.9(43.12) | -6.0(1.72) | 58.6(18.90) | -4.8(1.32) | 15.1(8.80) | 2.6(3.65) |
| F>25 | -172.5(67.70) | -4.8(2.70) | 50.6(29.51) | -5.9(2.08) | 7.9(13.75) | 14.2(5.76) |

†Fat percent, protein yield and SCS showed a significant interaction with parity, thus effects are included in the text

**Conclusions** Rate of increase in inbreeding in the population does not exceed the FAO guidelines 1% per generation (FAO, 1998). The significant quadratic effect of inbreeding on milk yield and protein percentage signifies that inbreeding has a more pronounced effect on these traits at higher levels. Scale of production is reflected in the lower than expected inbreeding depression, however results show that inbreeding significantly reduces milk, fat and protein yield and protein percent and increases fat percent and SCS.

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**064**

# Combining direct and correlated traits into an aggregate index for dairy cattle fertility

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**Introduction** In January 2006, ANAFI (Italian Holstein Breeders Association) introduced a genetic evaluation for fertility based on a multiple-trait animal model (Biffani *et al*., 2005), which included the following traits: days from calving to first insemination (DTFS), calving interval (CI), first-service non return rate to 56 d (NR56), angularity (ANG) and mature equivalent milk yield at 305 d (ME305). Breeding values have been subsequently combined in an aggregate index (T), with the breeding goal to increase conception rate (CR). This paper will show how the breeding values have been combined into an aggregate index. At the same time the efficiency of selecting on alternative aggregate indexes versus the official aggregate index is presented.

**Material and methods** The breeding values in T have been combined according to the methodology proposed by Schneeberger *et. al* (1992). The matrix with genetic (co)variance among traits in T and the matrix of genetic covariance between traits in T and trait in the breeding goal (table 1) were estimated with a multivariate animal model with all traits fitted simultaneously. In order to evaluate the effect of using different traits in T in terms of response ($R_T$) to and accuracy ($r_{TI}$) of selection, two alternative fertility indexes have been developed including one (CI) or three traits (CI, DTFS and NR56), respectively. Response to and accuracy of selection have been derived as described by Dekkers and Gibson (1998). The efficiency of selecting on an alternative index ($T_i$) versus the official index (T) can be computed as the ratio of accuracies ($r_{Tii}/r_{TI}$)

**Results** The traits included in the official (T) and alternative aggregate indexes ($T_1$, $T_2$), their relative emphasis and the effect of using different information sources on the response to and accuracy of selection are shown in table 1.

**Table 1** Relative emphasis on traits, response to and efficiency of selection for different fertility indexes [A]

| | | *Relative emphasis (%)* | | |
|---|---|---|---|---|
| | Indexes | T | $T_1$ | $T_2$ |
| Traits | | | | |
| ANG | | 7 | | - |
| CI | | 51 | 58 | 100 |
| DTFS | | 16 | 20 | - |
| NR56 | | 17 | 22 | - |
| ME305 | | 9 | | - |
| *Response in the breeding goal (CR)* | | 3 % | 2.7 % | 2.1 % |
| Efficiency of selection ($r_{Tii}/r_{TI}$) | | 1 | 0.95 | 0.75 |

[A] The efficiency is computed as the ratio of accuracies ($r_{Tii}/r_{TI}$)

Genetically, CI is the trait more (unfavorably) linked to the breeding goal and its relative emphasis in the aggregate index approaches 50%. The remainder is equally distribute among NR56 (17%), DTFS (16%) and the correlated traits (16%), ANG and MILK. Response to selection in the breeding goal using the official aggregate index (T) was 3% (per selection round), i.e. 0,5 genetic standard deviation. Indeed, the loss in total selection response was 10% and 30% when only three (T1) or one trait was included in the selection criteria. Using only CI, DTFS and NR56 as selection criteria decreases the efficiency of selection by 5%. When using only CI, the trait genetically most related to the breeding goal (CR), the efficiency of selection drops to 75 %.

**Conclusions** The main advantage in using direct and correlated traits to set up an aggregate index for fertility relies on the higher response to selection that can be attained. Selecting directly for CR would be the optimal solution, but this is not the case in Italy where CR can only be defined when a subsequent calving is observed or when the cow does not calve again. Using information on traits directly or indirectly related to CR seems to be more efficient even because they can be collected earlier. Selecting for fertility by means of an aggregate index allows not only to define a breeding goal that is economically important for the farmer but also to approximate what happens for a widely multi-genic trait like reproduction efficiency. Due to the unfavourable genetic correlation between fertility and production, a negative response to yield will be expected when selecting only for reproduction. Indeed, the aggregate index for fertility should be considered just as a part of the total breeding goal and afterward included in an overall selection index.

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# Genetic analyses of piglet survival and individual birth weight on first generation data of a selection experiment for piglet survival under outdoor conditions

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**Introduction** Peri- and postnatal mortality of piglets is reported to be around 20% and genetic improvement in piglet survival has great potential benefits in terms of animal welfare, economics and the environment. The indication of an unfavourable genetic correlation between litter size and survival in particular points to the importance of including piglet survival in those pig breeding programmes that currently only aim to increase litter size. Phenotypically, individual birth weight is closely associated with piglet survival (Roehe and Kalm, 2000). Genetic parameters for piglet survival traits and individual birth weight therefore need to be estimated in order to genetically improve piglet survival efficiency

**Materials and methods** Data on 5293 individual piglet observations from a selection experiment for piglet survival under outdoor condition were used in the genetic analyses. Piglets were from 414 litters of sows of a commercial crossbred line. The sires of piglets had high or average estimated breeding values (EBV) for maternal genetic effects contributing to percentage of survival between crossfostering to weaning. Traits analysed were survival at birth (SVB; complement of stillbirth), survival during the first day after birth (SVD1), survival during the entire nursing period (SVNP; including first day survival) and individual birth weight (IBW) including or excluding weights of dead born piglets. Multiple trait analyses were carried out using a threshold model for survival traits and a linear Gaussian model for birth weight. Fixed effects considered were selection group (high and average EBV), parity ($4^{th}$ or $5^{th}$ parity), gender, gestation length, month of farrowing and fostering (included only for the trait SVNP). As random effects, the direct genetic effects of the piglets and the litter effect were included in the model. Because no pedigree information for the dams was available, maternal genetic effects could not be estimated separately, and were therefore estimated in combination with maternal environmental effects in the litter effect. The analyses were based on a Bayesian approach using Gibbs sampling as implemented in the program THRGIBBSF90 (Misztal et al., 2002).

**Results** The means of SVB, SVD1 and SVNP were 93.5, 94.3 and 83.7%, respectively. Direct heritabilities of survival traits were significantly different from zero and their magnitudes were about one third of those estimates for birth weight (Table 1). Correlations among direct genetic effects of survival traits were low but favourable, as were genetic correlations between survival traits and birth weight. The posterior distributions of these estimates indicating that the probability of the correlations between IBW and SVB, SVD1 or SVNP are greater than zero were 0.83, 0.78 and 0.91, respectively. Excluding weights of dead piglets from the analysis reduced the genetic correlations between IBW and SVB, SVD1 or SVNP to 0.12, 0.10 and 0.20, respectively. The residual environmental correlations among survival traits were of high magnitude, those of birth weight with survival traits were small (Table 1). The proportion of the litter variance on the phenotypic variance were 0.22, 0.16, 0.11 and 0.21 for SVB, SVD1, SVNP and IBW (incl. Dead piglet's weight), respectively. These proportional litter effects were higher than the direct heritability for survival traits indicating that maternal genetic and environmental effects were more important than the piglet's direct genetic potential for these traits.

**Table 1** Direct heritabilities (diagonal), genetic correlations (above diagonal) and residual environmental correlations (below diagonal) for piglet survival traits and birth weight

| Trait | (SVB) | (SVD1) | (SVNP) | (IBW) |
|---|---|---|---|---|
| Survival at birth (SVB) | 0.08 (0.03) | 0.29 (0.25) | 0.36 (0.26) | 0.18 (0.19) |
| Survival at first day after birth (SVD1) | 0.75 (0.08) | 0.07 (0.03) | 0.40 (0.23) | 0.15 (0.18) |
| Survival during the nursing period (SVNP) | 0.76 (0.07) | 0.78 (0.03) | 0.08 (0.03) | 0.23 (0.17) |
| Birth weight (IBW; incl. dead piglet's weight) | 0.34 (0.03) | 0.36 (0.03) | 0.38 (0.03) | 0.20 (0.04) |

Standard errors in parenthesis

**Conclusions** The direct genetic effects of piglet survival traits were significantly different from zero, which indicates that improvement of piglets' genetic potential for survival is achievable. Using birth weight in the multiple trait model enhanced the estimation of direct genetic effects of survival. In addition, all genetic correlations between direct genetic effects of birth weight and survival traits were favourable. Moreover, as birth weight is measured on a continuous scale and has a moderate heritability, it has considerable value for genetic improvement of piglet survival. In conclusion, a combined selection for survival traits and an optimal birth weight may be most efficient to improve piglet survival.

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# Bayesian estimation of genetic parameters of litter size at birth in crossbred Mule ewes using repeatability and multiple trait threshold models

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**Introduction** The reproductive performance of ewes is a major factor influencing the economic success of lowland flocks. Even so reproductive traits have not often been included in sheep genetic improvement programs because of their low heritabilities compared with other performance traits like body weight and carcass merit. Litter size is one of the most important traits affecting reproductive performance. There are two important factors to be considered in the genetic analysis of litter size. First, several authors have suggested that litter size in different parities is controlled in part by different genes, and therefore should be treated as different traits. Second, ignoring the categorical nature of litter size and analysing it using a linear model does not account for its non-normal distribution. As a consequence, threshold models are likely to be more appropriate for the analysis of categorical traits (Gianola and Foulley, 1983). Therefore, the objective of the current study was to estimate the genetic parameters for litter size of Mule ewes using both repeatability and a multiple trait threshold models for repeated observations of litter size across four different parities.

**Materials and methods** Over a three year period (1998 to 2000), 1,500 Hill ewes comprising of two different breeds (Scottish Blackface and Hardy Speckled Face) were mated every year to 15 different Bluefaced Leicester rams at three experimental farms in Wales. The Mule ewe lambs produced were then distributed to three different evaluation sites in Wales, Scotland and England, ensuring a balance of the progeny of the 45 sires used across sites. The Mule ewes were crossed with terminal sire rams (Charollais, Suffolk and Texel in approximately equal proportions) in single sire mating groups each year for four parities. Data on 5,580 records of the first four parities of litter size at birth were available from 1,758 Mule ewes, lambing in the years from 2000 to 2005. The pedigree file consisted of 2,233 animals. The analyses were conducted assuming two different models, a repeatability threshold model (RM) and a multiple trait threshold model (MTM) for litter size in different parities. Furthermore, a simulation study, using the same pedigree structure and the estimated parameters of the Mule population, was carried out to quantify the appropriateness of the different models used for the analysis of litter size.

**Results** The estimated heritabilities of litter size in different parities using a MTM ranged from 0.12 to 0.18 (Table1) and were always higher than the heritability based on the RM (0.08). Using a RM the phenotypic proportion of the permanent environmental variance (0.14) of litter size was 1.75 times higher than the heritability. Genetic correlations between litter sizes of different parities were positive and ranged from 0.24 to 0.71, whereas corresponding environmental correlations ranged from 0.08 to 0.21 (Table 1). The accuracy of estimation of breeding values using different genetic models was assessed by simulation of 20 data sets based on the same pedigree structure and genetic parameters obtained from the multiple trait threshold analysis of the Mule ewe population. The simulated data sets were reanalysed using both the RM and MTM. Mean square errors and correlations between the true breeding values and posterior mean of the estimated breeding values were used for model comparison. The analysis using the multiple trait threshold model resulted in 2.9 to 7.8% lower mean square errors and 2.7 to 26.4% higher correlations between true and estimated breeding values in comparison to the analysis using a repeatability model for the different parities.

**Table 1** Posterior means, 95% highest posterior density regions (HPD95%) of residual correlations (above diagonal), heritabilities (on diagonal) and genetic correlations (below diagonal) for litter size at birth using a multiple trait threshold model (HPD95% intervals are in parenthesis).

| | Parity | | | |
|---|---|---|---|---|
| Parity | 1 | 2 | 3 | 4 |
| 1 | 0.16<br>(0.04 to 0.30) | 0.11<br>(-0.03 to 0.24) | 0.17<br>(0.02 to 0.31) | 0.17<br>(0.01 to 0.33) |
| 2 | 0.70<br>(0.24 to 0.99) | 0.17<br>(0.04 to 0.32) | 0.21<br>(0.08 to 0.32) | 0.08<br>(-0.06 to 0.22) |
| 3 | 0.54<br>(-0.15 to 0.99) | 0.71<br>(0.29 to 0.99) | 0.12<br>(0.01 to 0.24) | 0.17<br>(0.05 to 0.30) |
| 4 | 0.37<br>(-0.30 to 0.97) | 0.53<br>(0.05 to 0.95) | 0.24<br>(-0.46 to 0.83) | 0.18<br>(0.05 to 0.32) |

**Conclusions** These results indicate that the MTM was more appropriate than the RM to describe litter size, especially with the general decrease in the genetic correlation of litter size as the time interval between different parities increased. Based on all results, a MTM treating litter size in different parities as different traits is recommended.

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# Genetic progress in broiler traits – implications for body composition

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**Introduction** Genetic selection has dramatically increased the potential for growth, feed efficiency and yield in meat type broilers. It is a widely held belief that these improvements in genetic potential have produced a broiler that is lower in fat content. However, some recent work has implied that the Modern broiler is actually fatter than its counterpart of 30 years ago (Wang *et al*., 2004). The objective of this study was to explore the impact that selection for growth rate and yield has had on broiler body composition.

**Materials and methods** Three Modern lines (a female-MFL, a male-MML and an intermediate male line-MIML line; subjected to balanced selection for conventional broiler characteristics and welfare traits) and their 1972 Control line equivalents; CFL, CML and CIML (discrete populations, randomly selected to maintain the characteristics of the 1972 commercial broiler) were fed diets with three levels of balanced protein relative to the breeder's recommended level (Ross Management Manual, 2002) to induce different levels of fatness. The levels were 80%; with 189, 168 and 147 g/kg CP for Starter (0-10d), Grower (11-28d) and Finisher (28d+), 100%; with 221, 192 and 172 g/kg CP respectively and 120% with 261, 224 and 199 g/kg respectively. Birds were reared in a windowless, temperature controlled house in 180 pens, each with 30 birds at day old. Stocking density was kept below 34kg/$m^2$ at all times. Body weights were measured at weekly intervals up to 42 days of age in the Modern lines and up to 77 days in the Control lines, as the Control lines had a much slower growth rate this ensured that similar weights for comparison were achieved. Six birds per pen were removed for processing at 28, 35 and 42 days (Modern lines) and at 42, 56, 70 and 77 days (Control lines); these ages were chosen to ensure that birds from both lines were killed at similar weights. Processing performance was corrected by interpolation to a common weight of 2kg. In addition two birds per pen, from the 100% balanced protein diet only, were sent for carcass fatness assessment (ether extract content of eviscerated carcass, EE); these birds were removed 35 days for the Modern lines and 77 days for the Control lines. It was predicted that at these ages both Modern and Control lines would be approximately 2.4kg however actual weights averaged 2.0kg for the Modern lines and 2.5kg for the Control lines. Total fat content of the eviscerated carcass (TCF) was calculated as EE plus the weight of abdominal fat pad. Data were analysed in Minitab using a General Linear Model.

**Results** Table 1 shows the body composition of the lines on the 100% balanced protein diet. Modern lines were significantly heavier (P<0.001), had a significantly higher eviscerated yield (P<0.001), significantly more breast meat (P<0.001) and a lower amount of abdominal fat (AFP, P<0.001), than Control lines, both at 42 days and at 2kg. A similar pattern was seen on the 80% and 120% balanced protein diets (data not shown). Total body fat content at ~2.3kg on the 100% balanced protein diet was significantly lower in the Modern lines than in the Control lines (P<0.001). Figure 1 shows that the Modern lines were more responsive in breast meat yield to dietary protein content than the Control lines, showing on average a 3.6% increase in breast meat at 2kg when protein was increased from 80% to 120% of recommended levels.

**Table 1**. Liveweight (LWT), eviscerated yield (EY), breast meat yield (BM), abdominal fat pad (AFP) and total carcass fat (TCF) in Modern (2005) and Control (1972). Data are means of males and females

| | | 42 days | | | 2kg | | | ~2.3kg |
|---|---|---|---|---|---|---|---|---|
| Line | | LWT | EY | BM | EY | BM | AFP | TCF |
| | | g | g/kg | g/kg | g/kg | g/kg | g/kg | g/kg |
| Modern | MML | 2880 | 709 | 189 | 692 | 186 | 14 | 153 |
| | MFL | 2449 | 681 | 163 | 668 | 162 | 19 | 173 |
| | MIML | 2455 | 689 | 189 | 668 | 174 | 16 | 156 |
| Control | CML | 1204 | 634 | 103 | 654 | 112 | 31 | 269 |
| | CFL | 1216 | 642 | 105 | 652 | 109 | 36 | 269 |
| | CIML | 1229 | 628 | 108 | 659 | 118 | 32 | 248 |
| *Std error of mean* | | *51.0* | *8.2* | *2.4* | *2.7* | *2.1* | *1.4* | *7.6* |

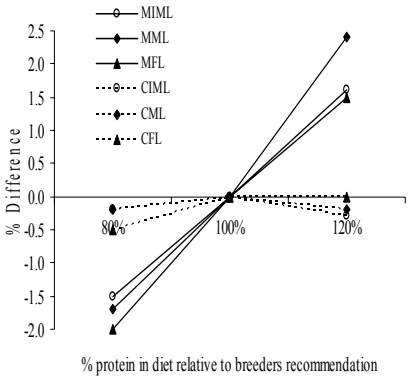


**Figure 1** Response of breast meat yield at 2kg in Modern lines and Control lines to dietary protein content

**Conclusions** Genetic progress has resulted in significant changes in the body composition of the broiler. These data show that the Modern lines were significantly heavier with a higher proportion of breast meat (almost 54% higher on a weight basis) and a lower amount of body fat than the Control lines (Modern lines were almost half as fat as Control lines). In addition, Modern lines were shown to be more responsive to dietary protein than Control lines. The results from this study are in direct contrast to the work of Wang et al. (2005) which concluded that Modern broilers are fatter than their counterparts of 30 years ago.

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# Effect of index selection in GGP lines on cocoon traits of silkworm commercial hybrids

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**Introduction** The general aim of selection in animal breeding is to acquire new generation of animals, which under future economic conditions of production system are more efficient than the present generation. In selection index method, genetic trend of important traits is directed based on their weights in economic efficiency of production system to acquire maximum improvement in economic merit (Mirhosseini *et al.*, 2005). Silkworm commercial egg is produced by crossing between Japanese and Chinese origin parent stocks. There are three steps in the cycle of egg production including GGP (great grand parent), GP (grand parent) and P (parent stock). Breeding programs along with high selection pressure are used only in GGP lines which are typically much smaller in size than other stocks. The objective of this study was to evaluate effect of six generations of index selection in the GGP lines on performance of commercial silkworm hybrids.

**Materials and methods** The present investigation has been carried out on four commercial silkworm lines produced in Iran's Silkworm Research Center (ISRC) including Chinese origin lines of 110, 154 and Japanese origin lines of 107, 153. The data used were approximately 8800 records for cocoon characteristics including cocoon weight, cocoon shell weight and cocoon shell percentage. After estimating (co)variance components by REML method, additive genetic values of animals were predicted using Best Linear Unbiased Prediction (BLUP). After constructing selection index, selection ensued for six generations. 110×107, 107×110, 153×154 and 154×153 hybrids were produced using mating between GGP lines being affected by six generations of index selection after GP and P steps. Similar hybrids as control groups were produced from not-selected lines to be compared with selected hybrids. Hybrids were reared in four replications for each group in which 30 male and 30 female cocoons were individually recorded per replication. The experiment was replicated in three rearing seasons. Statistical analysis was performed as factorial design considering hybrid, group, sex and season factors as fixed effects under generalized linear models.

**Results** In table 1 average of cocoon wt, cocoon shell wt and cocoon shell % is shown in 4 commercial hybrids for selected and non-selected (control) groups. Significant difference was observed between cocoon characters of hybrids obtained from selected lines and non-selected (control) lines. Selected group compared to control one showed 1.23, 7.84, 7.62, 4.94 % increase in mean cocoon wt, 7.48, 13.75, 12.47, 11.99 % increase in mean cocoon shell wt and 6.23, 5.73, 4.42, 3.92 % increase in mean cocoon shell percentage for 110×107, 107×110, 153×154 and 154×153 hybrids, respectively. Index selection on GGP stocks resulted in greater gain in cocoon shell wt than other traits, while cocoon shell percentage in the hybrids indicated lower phenotypic gain due to index selection. Use of selection index program resulted in more gain of cocoon wt and cocoon shell wt in 107×110 and 153×154 hybrids, but more gain of cocoon shell percentage in 110×107 and 107×110.

**Table 1** Average of cocoon traits (± standard deviation for 720 records) in 4 silkworm commercial hybrids obtained from selected GGP lines and non-selected (control) GGP lines[†]

| | Cocoon wt (g) | | Cocoon shell wt (g) | | Cocoon shell % | |
|---|---|---|---|---|---|---|
| Hybrid | selected | control | selected | control | selected | control |
| 110×107 | $1.649^{c}$±0.293 | $1.629^{c}$±0.289 | $0.345^{c}$±0.067 | $0.321^{b}$±0.069 | $21.15^{b}$±3.11 | $19.91^{c}$±3.21 |
| 107×110 | $1.719^{b}$±0.287 | $1.594^{d}$±0.309 | $0.364^{b}$±0.061 | $0.320^{b}$±0.066 | $21.41^{ab}$±2.57 | $20.25^{b}$±2.79 |
| 153×154 | $1.907^{a}$±0.328 | $1.772^{b}$±0.345 | $0.406^{a}$±0.067 | $0.361^{a}$±0.074 | $21.51^{a}$±2.65 | $20.60^{a}$±2.68 |
| 154×153 | $1.911^{a}$±0.330 | $1.821^{a}$±0.333 | $0.411^{a}$±0.069 | $0.367^{a}$±0.071 | $21.19^{b}$±2.87 | $20.39^{ab}$±2.68 |

[†]Difference between selected and control groups was significant at 0.05 level for all the hybrids. In each column, Means with the same letter are not significantly different (alpha=0.05)

**Conclusions** The results obtained demonstrated that selection index procedure is clearly advantageous to be used in GGP lines achieving topmost gains in cocoon characteristics of hybrids. Therefore, selection index programme could be efficiently performed to improve genetic-economic performance of the lines. 107×110 and 153×154 non-selected hybrids had lower mean cocoon wt and cocoon shell wt than reciprocal ones, while 107×110 and 153×154 selected hybrids indicated higher gain for these traits than reciprocals. This shows that these traits are affected by sexuality so that genetic gain in Japanese lines is greater in females and in Chinese lines is larger in males (Kang *et al.*, 2003). Knowledge of actual economic response to selection can help to silkworm breeder to use high economically efficient lines in hybridization program to maximize cocoon producers' profitability in the future economic conditions.

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# Prospects for genetic improvement of milk production traits of Sahiwal cattle in Kenya

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**Introduction** The primary emphasis of the long-term Sahiwal cattle breeding programme is to increase milk yield by selecting cows based on their performance in first three lactations. It is therefore important to have knowledge on the extend of additive genetic variance and genetic parameters for these traits. Genetic and phenotypic parameter estimates normally apply directly to the specific population and environment from which the data were collected. In the Sahiwal cattle in Kenya, very little is known about the genetic variation of milk production traits and their genetic relationships. Furthermore, genetic and phenotypic parameter estimates for the Sahiwal cattle based on multivariate animal model are scarce. This paper presents estimates of variance components and genetic parameters for milk production traits using trivariate animal model.

**Materials and methods** The data set analysed consisted of 6365 lactation records from 257 sires and 2894 cows born between 1963 and 2000 at the National Sahiwal stud, Naivasha Kenya. These cows calved down between 1965 and 2003. Details of the data source, climate and herd management have been described (Ilatsia et al. 2006). Milk production traits considered were lactation milk yield (LMY, kg) and lactation length (LL, days) in the first three lactation. Variance components, heritability, genetic and phenotypic correlations were estimated using a trivariate animal model. Lactation milk yield and LL in first three were considered as distinct traits and the analysis carried out using the DFREML software package (Meyer, 1989). Fixed effects fitted in the model were year-season of calving and age at calving. Mixed model equations were solved iteratively and the simplex procedure was used to locate the maximum of the log-likelihood.

**Results** Table 1 shows heritability estimates, genetic and phenotypic correlative for LMY and LL in the three lactations. Heritability estimates were moderate for LMY (ranges of 0.32 to 0.45) and LL (ranges 0.26 to 0.34). The higher heritability estimates are associated with substantial large additive genetic variance associated milk production traits (Table 1). Furthermore multivariate models effectively account for selection across lactation and better adjust for environmental factors. These findings are consistent with what has been reported for Sahiwal cattle in Pakistan (Dahlin et al. 1998). Genetic correlations among milk production traits were high while phenotypic correlations were correspondingly lower. The high genetic correlations indicate that selection for improved performance in milk production in the first lactation would result in genetic progress in later lactations as well. Similar findings have been reported for Sahiwal population in Pakistan (Dahlin et al., 1998).

**Table 1** Estimates of heritability, genetic and phenotypic correlations additive ($\sigma_a$), and phenotypic ($\sigma_p$) standard deviations of milk production traits

| Trait | Lactation[1] | | | Variances | |
|---|---|---|---|---|---|
| For lactation no. | 1 | 2 | 3 | $\blacklozenge_a$ | $\blacklozenge_p$ |
| LMY (kg) | | | | | |
| 1 | **0.32** | 0.40 | 0.36 | 257.8 | 458.7 |
| 2 | 1.00 | **0.45** | 0.57 | 297.6 | 441.7 |
| 3 | 0.98 | 1.00 | **0.41** | 290.7 | 452.2 |
| LL (days) | | | | | |
| 1 | **0.26** | 0.32 | 0.30 | 25.5 | 49.4 |
| 2 | 0.99 | **0.31** | 0.39 | 28.1 | 50.3 |
| 3 | 0.92 | 0.99 | **0.34** | 29.6 | 50.3 |

[1]Genetic correlations (below diagonal) phenotypic correlations (above diagonal)

**Conclusions** Milk production traits are moderately heritable in the Sahiwal cattle in Kenya and this offers an opportunity for genetic improvement through selection. Genetic improvement in later lactation could be achieved by selecting cows based on milk performance in early lactations. This would result in faster genetic gain since selection decisions would be made early.

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# Estimation of genetic correlation between milk production and fat yield in different climates of Iran

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**Introduction** Performance of one genotype in similar climates is approximately the same but if this genotype is introduced into a different climate, its performance will be affected, based on Nizamani and Berger (1996). The function that relates phenotype to environment is unique for each genotype. Hence, the response to changes in environment may vary from one genotype to the other, based on Mulder et al. (2004). Different selection responses between environments are generally attributed to two types of genotype by environment interaction. The first type occurs when the genetic correlation between performances in two environments is substantially less than 1.0, indicating a genetic difference basis for the trait in the two environments. The second type of genotype by environment interaction results from heterogeneous variances, based on Ojango and Pollott (2002). The goal of this study was to estimate genetic (co)variances, environmental variances, and genetic parameters of milk production and fat yield among different environments in Iran to determine variables that are useful indicators of genotype by environment interaction.

**Materials and methods** First – lactation records on milk production and fat yield for 102371 Holstein cows produced 1991-2001, were used in this study. The subset of data used in this study was restricted to records of 305-d and twice daily cows were classified before 36 mo of age and, at most, 18 mo after calving. Only cows with both milk production and fat yield information were included and these records were grouped in 5 different climates, including dry, semi-dry, mediterranean, humid and semi-humid, on the basis of Extended De martonne method, selected sires had records in most of these climates. Normality of distribution of data was examined by using SAS 6.2 software and appropriate statistical model for each trait was determined by Proc GLM. Genetic (co)variances, residual variances and correlation coefficient were estimated for milk production and fat yield in different climates of Iran by multiple-trait animal model and restricted maximum likelihood (REML) algorithm.

**Results** The results of ANOVA analyses showed that the effects of herd-year-season (HYS) as fixed factors and calving age as covariate variable for milk production were significant ($P<0.01$) and for fat yield fixed and auxiliary factors were significant at the 1% level in all climates, except for age of birth auxiliary variable in dry climate that was significant at the 5% level. The mean of milk production for semi-dry, mediterranean, dry, Semi-humid and humid climates were 6378.05, 5967.28, 5854.18, 5527.53, and 4873.43, respectively and the mean of fat yield for semi-dry, dry, mediterranean, semi-humid and humid climates were 196.60, 182.35, 174.54, 167.59 and 159.46, respectively. In Table 1 heritability of milk production and fat yield is shown for the five climates.

**Table 1** Heritability of milk production and fat yield in different climates

| Climate/ Trait | Dry | Semi-dry | Mediterranean | Humid | Semi-humid |
|---|---|---|---|---|---|
| Milk production | 0.28 | 0.30 | 0.24 | 0.29 | 0.26 |
| Fat yield | 0.12 | 0.26 | 0.22 | 0.23 | 0.14 |

**Table 2** Genetic correlation of milk production and fat yield between different climates

| Climate | Dry | Semi-dry | Mediterranean | Humid | Semi-humid |
|---|---|---|---|---|---|
| Dry | 1 | 1 | 0.99 | 0.84 | 0.96 |
| Semi-dry | 1 | 1 | 0.98 | 0.73 | 0.98 |
| Mediterranean | 0.90 | 0.90 | 1 | 0.66 | 1 |
| Humid | 0.26 | 0.16 | 0.65 | 1 | 1 |
| Semi-humid | 0.96 | 0.84 | 0.78 | 0.68 | 1 |

Elements under the diagonal are related to fat yield and elements above it are related to milk production.

As showed in Table 2, genetic correlation coefficients for milk production between humid and dry, semi dry and mediterranean climates were lower than 0.9 that suggested significant genetic and environment interaction. In most of cases, genotype by environment interaction (G×E) between humid climate and other climates for fat yield were significant and the lowest interaction was derived between humid and semi-dry climates (0.16).

**Conclusion** The lowest genetic correlation of milk production was related to humid and mediterranean climates and lowest genetic correlation of fat yield was related to humid and semi-dry climates indicating the highest genotype by environment interaction (G×E) between these climates. Because of positive correlation between these two traits, results were somewhat similar.

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# Estimation genetic and phenotypic correlations between weight traits pre and post weaning weight in Baluchi sheep

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**Introduction** Baluchi is the most common native breed of sheep in Iran, comprising 30% of the sheep population. This breed is native to the eastern part of the country, which has a dry and hot climate. The animals have had to adapt to the harsh environment. In order to improve efficiency, the performance of the animals for economic traits is used to estimate genetic parameters and selection in some nucleus flocks. The genetic and phenotypic correlations between weight traits have been reported for different breeds. The objective of this investigation is to estimate the genetic parameter and genetic and phenotypic correlations between weight traits.

**Materials and methods** The data were collected from a Baluchi flock in the Animal Breeding Station of Abbas Abad in northeast Iran. The data were for birth weight (BW), weaning weight (WW), six (6MW), nine (9MW), twelve (12MW) month weight, average daily gain from birth to weaning weight (ADGPreW), average daily gain from weaning to yearling weight (ADGPosW) (Table 1). Age of dam (10 level), sex (2 level), birth type (3 level), year (12 level) and month (3 level) of birth were considered as fixed effects and age of lamb on the day of recording was used as a covariate.

**Table 1** Summary of characteristic of weight traits ( kg )

| | BW | WW | 6MW | 9MW | 12MW | ADG PreW | ADG PosW |
|---|---|---|---|---|---|---|---|
| Mean | 4.31 | 22.4 | 31.7 | 34.4 | 38.6 | 0.240 | 0.057 |
| Standard deviation | 0.68 | 4.4 | 5.6 | 5.7 | 6.3 | 0.047 | 0.019 |
| Coefficient of variation (%) | 15.7 | 19.6 | 17.6 | 16.5 | 16.3 | 19.5 | 33.3 |
| Number of records | 5913 | 5146 | 4434 | 3671 | 3716 | 5145 | 3712 |
| Number of fixed effects + co variable | 5 | 5+1 | 4+1 | 4+1 | 4+1 | 5+1 | 4 |

Genetic and phenotypic correlations were estimated by REML method under a two trait Animal Model:
$Y = Xb + Za + e$ where Y is the vector of observations, b is the vector of fixed effects, a is the vector of random additive direct genetic effects, and e is the vector of random residual effects. X, Z are known incidence matrices connecting the observations to the respective fixed and random.

**Results** Estimates of heritabilities for traits one and two and genetic and phenotypic correlations between weight traits before and after weaning are presented in Table2.

**Table 2** Heritabilities for traits one and two and genetic and phenotypic correlations

| Trait 1 | Trait 2 | $h^2_1$ | $h^2_2$ | $r_g$ | $r_p$ |
|---|---|---|---|---|---|
| BW | 9MW | 0.44 | 0.31 | 0.56 | 0.31 |
| BW | YMW | 0.45 | 0.37 | 0.59 | 0.31 |
| WW | 9MW | 0.32 | 0.38 | 0.92 | 0.50 |
| WW | YMW | 0.32 | 0.40 | 0.94 | 0.61 |
| ADG-PreW | ADG-PostW | 0.28 | 0.09 | -0.07 | -0.17 |
| 9MW | YMW | 0.30 | 0.33 | 0.95 | 0.82 |

Heritabilities for all of weight traits were high but for ADG-PostW was lower than 0.1. Genetic and phenotypic correlations between birth weight with nine and twelve-month weight were lower compared with these values between weaning weight with 9MW and 12MW but the values between 9MW and 12MW were highest. These Correlations between ADG-PreW and ADG-PostW were negative. These results are in agreement with other reports (Bromley et al. 2000. Hanford, K. J. et al. 2002).

**Conclusions** Heritabilities, genetic and phenotypic correlations between weight traits can be an important parameter for genetic improvement. Selection of replacement lambs in the flock according to these values can be expected to bring about a response in the selected traits. These results suggest that using records of weight at early ages could select animals that have maximum weight at a later age.

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# Derivation of economic values for dairy cattle in Iran and comparison to other countries

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**Introduction** Index selection is the most effective selection strategy to improve total merit. Index selection requires the economic values (EVs) of the traits in the breeding goal and the genetic parameters of the traits in the breeding goal and in the index (Hazel and Lush, 1942). According to this theory, in most countries a national selection index has been developed for dairy cattle (Miglior et al., 2005). In Iran a national selection index has not yet been developed. The first objective of this study was, therefore, to estimate economic values for production traits (milk, fat and protein yield) and longevity for Holsteins in Iran. The second objective was to compare the proposed Iranian selection index with selection indices of other countries in the world.

**Materials and methods** A profit equation was used to describe the revenues and costs of the Holstein dairy cattle industry in Iran. Production parameters and economic data were collected from two large Holstein dairy cattle farms representing the dairy farming situation in Iran. Revenues came from milk, pregnant heifer, culled cow and manure sales. Costs were divided into feed and non-feed costs. Non-feed costs included costs related to labor, veterinary services, insemination, housing, fuel and insurance. Economic values were derived as the first partial derivative of the profit equation at the population mean of each trait. Relative emphasis was calculated using equation (1).

(Equation 1) $$RE_i = (EV_i \times GSD_i / \sum_{i=1}^{4} (EV_i \times GSD_i)) \times 100$$

Where $RE_i$,, $EV_i$ and $GSD_i$ are relative emphasis, economic value and genetic standard deviation for the trait, respectively.

**Result** Table 1 shows absolute and relative economic values and the relative emphasis of 4 traits in Iranian national selection index, Lifetime Net income Index (LNI), which were calculated in 2006 based on profit as a breeding perspective. In this study economic values were derived for production traits (milk, fat and protein yields) and longevity, 0.1, 0. 9, -0.2 and 6.2, respectively. Due to higher costs than revenues for protein yield in the Iranian milk pricing system, the economic value of protein yield is negative. In the future, the revenues of protein may increase and therefore the economic value of protein yield was set to zero. The absolute economic value of longevity was bigger than yield traits. LNI does not include health, reproduction, and workability traits. This was different from other countries. Therefore, a higher emphasis must be used for longevity because of playing a role as a summarizing trait.

**Table 1** Economic values and relative values of 4 traits in Lifetime Net income Index (LNI) in 2006

| Trait | Genetic standard deviation[1] | Absolute EV($) | Relative EV[2] | Relative emphasis (%) |
|---|---|---|---|---|
| Milk yield (Kg) | 561.7 | 0.1 | 0.02 | 59.7 |
| Fat yield (Kg) | 14.9 | 0.9 | 0.14 | 14.3 |
| Protein yield (kg) | 14.0 | -0.2 | -0.03 | -3.0 |
| Longevity (Month) | 3.5 | 6.2 | 1 | 23.1 |

[1] From Dadpasand Taromsari et al, 2006. [2] Relative EVs are based on EV of longevity which is the biggest.

Table 2 shows the relative emphasis on traits in national selection indices. Most semen and embryos used in Iran, are imported from USA and Canada. These countries have no emphasis on milk yield but have high emphasis on protein and fat yields and a lower emphasis on longevity. Importation of semen and embryo's from these countries, therefore, may not be optimal from a genetic point of view.

**Table 2** Relative emphasis on longevity and milk production traits in national selection indices

| Country | Index | Milk yield | Fat yield | Protein yield | Longevity | Other traits |
|---|---|---|---|---|---|---|
| Canada1 | LPI2 | - | 14.3 | 42.7 | 7.6 | 35.4 |
| Great Britannia1 | PLI3 | -16.4 | 9.5 | 49.1 | 15 | 10 |
| Islamic Republic of Iran | LNI | 59.7 | 14.3 | -3 | 23.1 | 0 |
| United States of America1 | Net Merit | - | 22 | 33 | 11 | 34 |

[1]Obtained from Miglior et al, 2005 [2] LP1 = Lifetime profit index [3] PLI = Profitable lifetime index

**Conclusion** In the future, protein content of milk may become more important, therefore the economic value of protein content was set to zero. LNI was suggested to the Iranian Animal Breeding Center (IABC) to select cows and bulls for progeny test based at current economic situation.

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# Effects of rearing environment, zinc oxide and butyrate upon gut structure in the newly weaned pig

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**Introduction** Outdoor reared pigs are said to suffer from less of a growth check post weaning than their indoor reared counterparts, Payne *et.al* (2003). Zinc oxide (ZnO) is often used to reduce the incidence of post weaning scours and the use of sodium butyrate as an additive in weaner pig diets has been shown to improve some aspects of small intestine structure (Miller *et.al*, 2006). The purpose of this experiment was to investigate the effects of butyrate supplemented weaner diets with or without a ZnO background (3.1 or 0g/kg ZnO) upon gut structure immediately post weaning in both indoor and outdoor reared pigs. We hypothesised that piglets reared outside would have a more developed gut than indoor reared pigs when weaned at 4 weeks and that the presence of ZnO and butyrate would further enhance gut development.

**Materials and methods** Sixty-four indoor and sixty-four outdoor reared pigs of the same geneotype (JSR Healthbred) were weaned at 27 ± 0.17 (± s.e.m) days of age and 8.6 kg and 7.8 kg ±0.17 liveweight respectively into fully slatted weaner pens balancing for liveweight, sex and litter across treatments. Piglets were offered *ad libitum* access to diets (16.2 MJ DE/kg, 1.6g/kg lysine) containing either 0 or 1.5g/kg esterified butyrate and supplemented with either 0 or 3.1g/kg ZnO. Diets were fed for 13 days post weaning. Pigs were killed at d6 or d13 and intestinal morphology at 0.25 and 0.75 along the small intestine measured. Data were analysed as a 2x2x2 factorial using the GLM procedures of Minitab 12.2.

**Results** Rearing environment had the greatest effect upon the structure of the small intestine in the distal region, in particular there was an increase in the number of crypt located goblet cells (P≤0.05) on both d 6 and 13 post weaning in the outdoor reared pigs. Supplementing the diets with ZnO enhanced development of the small intestine structure in the proximal and distal region on day 6 and the proximal region on day 13 post weaning (Table 1), whilst rearing environment was shown to have no influence upon development of the proximal small intestine. The inclusion of butyrate had no effect upon of gut structure on either day 6 or 13 post weaning.

**Table 1** Effect of rearing environment and ZnO upon villus height (VH), crypt depth (CD), villus goblet cell number (GC) and crypt goblet cell number (GC) on days 6 and 13 post weaning.

| | ZnO | | | | Rearing Environment | | | | P value | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Yes | s.e.m | No | s.e.m | In | s.e.m | Out | s.e.m | ZnO Effect | In/Out Effect | Interaction |
| Day 6 | | | | | | | | | | | |
| VH 0.25 (μm) | 294 | 9.4 | 239 | 10.4 | 263 | 10.0 | 269 | 10.0 | 0.001 | NS | NS |
| CD 0.25 (μm) | 222 | 5.3 | 232 | 5.9 | 224 | 5.6 | 229 | 5.7 | NS | NS | NS |
| Villus GC (0.25) | 4.3 | 0.36 | 3.2 | 0.40 | 4.3 | 0.38 | 3.3 | 0.38 | 0.039 | NS | NS |
| VH 0.75 (μm) | 267 | 9.2 | 223 | 9.2 | 248 | 9.7 | 243 | 9.2 | 0.001 | NS | NS |
| CD 0.75 (μm) | 208 | 4.8 | 212 | 4.8 | 196 | 5.1 | 224 | 4.8 | NS | 0.001 | NS |
| Crypt GC (0.75) | 13.6 | 0.78 | 13.1 | 0.78 | 11.8 | 0.83 | 14.8 | 0.78 | NS | 0.012 | NS |
| Day 13 | | | | | | | | | | | |
| VH 0.25 (μm) | 341 | 11.3 | 290 | 12.7 | 314 | 12.1 | 318 | 13.1 | 0.005 | NS | NS |
| CD 0.25 (μm) | 237 | 7.5 | 226 | 8.4 | 226 | 8.0 | 236 | 8.7 | NS | NS | NS |
| Villus GC (0.25) | 6.4 | 0.60 | 3.8 | 0.68 | 4.9 | 0.65 | 5.3 | 0.70 | 0.006 | NS | NS |
| VH 0.75 (μm) | 314 | 12.2 | 288 | 10.8 | 277 | 12.1 | 325 | 12.0 | NS | 0.011 | NS |
| CD 0.75 (μm) | 195 | 6.5 | 208 | 5.8 | 198 | 6.4 | 204 | 6.4 | NS | NS | NS |
| Crypt GC (0.75) | 13.7 | 0.69 | 13.4 | 0.61 | 12.3 | 0.69 | 14.8 | 0.68 | NS | 0.019 | NS |

**Conclusions** The outdoor reared pigs showed a better developed distal gut on both days 6 and 13 post weaning compared to the indoor reared pigs. This is in agreement with our hypothesis that outdoor reared pigs would show greater gut development, possibly due to the opportunities to forage and consume substrates prior to weaning. ZnO supplementation was also shown to enhance the structure of the proximal part of the small intestine on both days as hypothesised, however esterified butyrate showed no benefit towards enhancing gut structure.

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# Neonatal piglet survival indicators in an outdoor system

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**Introduction** The current estimate of live-born piglet pre-weaning mortality in UK outdoor systems is 11% (MLC, 2006), representing a major welfare and economic concern. The majority of deaths occur in the first few days of life when the piglets are at their most vulnerable. Selecting for high survival in piglets, may allow breeding of animals better able to survive and better suited to alternative and less restrictive systems than the farrowing crate. The aim was to develop and measure behavioural and physiological indicators of neonatal survival, and to determine treatment effects in populations of piglets selected for high survival and control lines farrowing in an outdoor system.

**Materials and methods** Thirty-eight outdoor sows were served by boars selected for a piglet survival trait, producing two treatment groups; 19 high survival litters (HS), 19 control litters (C). Behavioural and physiological measurements were taken from piglets throughout the neonatal period. These included birth weight (BW), crown-rump length (CRL), ponderal index (PI) (birth weight/crown-rump$^3$), body mass index (BMI) (birth weight/crown-rump$^2$), rectal temperatures at birth, 1h and 24h after birth, 24h blood glucose, gender, birth order (BO), birth interval (BI), farrowing duration (FD) and behavioural landmarks such as latencies from birth to reach the udder, teat and to first ingest colostrum (suckle). Post-mortem analyses were conducted on all dead piglets. Statistical analysis used a Generalised Linear Mixed Model (GLMM), allowing a binomial structure and using a Logit transformation (SAS version 9.3.1). Two models were adopted, the first comparing surviving piglets with still-born piglets (still-born model) and the second comparing surviving piglets with piglets dying post-partum (live-born mortality model). Univariate and multivariate analyses were performed on both these models.

**Results** Total mortality was 21.0% in C litters and 14.4% in HS litters ($\chi^2_1 = 3.754$, P=0.05). Total live-born mortality was 14.6% in C litters and 12.2% in HS litters ($\chi^2_1 = 0.762$, P=0.383). Univariate analyses for the two models identify specific factors as significant for explaining mortality (Table 1 (still-born) and 2 (live-born)).

**Table 1**: Univariate GLMM comparing surviving piglets (n=412) with those still-born (n=26)

| Factor | Surviving (±SE) | Still-born (±SE) | F-stat |
|---|---|---|---|
| BW (g) | 1553 (16.7) | 1234 (88.8) | 46.99 *** |
| CRL (cm) | 28 (0.14) | 29 (0.75) | 23.68 *** |
| PI | 75.34 (0.86) | 50.50 (2.74) | 130.19 *** |
| BMI | 20.46 (0.18) | 14.44 (0.78) | 152.01 *** |
| BO | 7 (0.21) | 12 (0.71) | 81.46 *** |
| BI (mins) | 17 (1.19) | 33 (9.91) | 26.68 *** |
| FD (mins) | 117 (4.35) | 449 (53.9) | 69.49 *** |

**Table 2**: Univariate GLMM comparing surviving piglets (n=412) with those live-born piglets subsequently dying (n=64)

| Factor | Surviving (±SE) | Dying (±SE) | F-stat |
|---|---|---|---|
| BW (g) | 1553 (16.7) | 1249 (53.2) | 69.73 *** |
| CRL (cm) | 28 (0.14) | 25 (0.43) | 43.08 *** |
| BMI | 20.46 (0.18) | 18.67 (0.57) | 21.08 *** |
| 24h glucose (mmol) | 4.72 (0.10) | 3.50 (0.27) | 25.55 *** |
| Birth temp (°C) | 37.45 (0.06) | 36.90 (0.22) | 25.75 *** |
| 1h temp (°C) | 37.66 (0.06) | 36.88 (0.29) | 23.70 *** |
| 24h temp (°C) | 38.43 (0.05) | 37.69 (0.27) | 21.94 *** |
| Suckle (mins) | 28 (2.03) | 29 (7.34) | 3.98 * |

Multivariate analysis for the still-born model implicated ponderal index ($F_{1,409} = 120.13$, P<0.001) and farrowing duration ($F_{1,434} = 63.96$, P<0.001) as the most important survival factors. In the live-born mortality model, birth weight ($F_{1,472} = 17.46$, P<0.001), 1h rectal temperature ($F_{1,423} = 6.15$, P<0.01), 24h rectal temperature ($F_{1,431} = 6.52$, P<0.01), 24h blood glucose ($F_{1,431} = 14.17$, P<0.001) and gender ($F_{1,475} = 17.44$, P<0.001) were the most important survival factors.

**Conclusions** HS litters experienced less total mortality than C litters, which was mostly accounted for by the number of still-born piglets. A piglet of higher birth weight, adequately thermoregulating and suckling successfully (indicated by 24h blood glucose) was more likely to survive the vulnerable neonatal period, with male piglets being more at risk than females during this time. Despite the significance of birth weight in the live-born mortality model, with respect to surviving *in utero* and during the farrowing process, body shape was more important. There is potential to utilise these survival indicators to breed for decreased total piglet mortality and this improvement in survival may allow the introduction of less restrictive farrowing systems.

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**References:** Meat Livestock Commission, 2006. *Pig yearbook* 2006

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# Both outdoor and indoor reared piglets respond to antimicrobial supplementation in post-weaning diets regardless of weaning age

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**Introduction** Outdoor piglets are reported to eat more readily post-weaning than indoor pigs and show improved post weaning performance (Cox and Cooper, 2001). They are therefore likely to be less reliant on the inclusion of antimicrobial agents in the diet post weaning to enhance performance. Similarly piglets which are older at weaning should also be more resilient and less likely to benefit from antimicrobial agents in the feed. In this experiment we compared piglets reared indoors with those reared outdoors at two different weaning ages to determine whether performance was less dependent on dietary antimicrobial additives in the outdoor-reared older pig.

**Materials and methods** One thousand one hundred and eight pigs (Large White x Landrace) were born and reared either indoors or outdoors and weaned at either 4 or 6 weeks of age into 160 flat deck pens to give 20 replicates of 6 to 8 pigs per pen per treatment. Piglets were offered *ad libitum* access to diets (15 MJ DE/kg, 1.3 g lysine/kg) containing no antimicrobials or a combination of 3.1 g zinc oxide /kg and 40 mg avilamycin/kg for 14 d following weaning. Feed intake was measured daily (ADFI). Average daily gain (ADG) and Feed Conversion Ratio (FCR) were calculated on a pen basis for days 1 to 14. Data were analysed as a 2x2x2 factorial using the GLM procedures of Minitab 13.0. Weaning weight differed between treatments and was therefore used as a covariate in subsequent statistical analysis except for FCR data where it was not significant and 8 week weight.

**Results** Over the 2 weeks after weaning, rearing environment did not affect feed intake but outdoor pigs used feed more efficiently to give faster weight gain ($P<0.05$). Older pigs ate more and grew faster ($P<0.001$) but used their feed with similar efficiency to younger weaned pigs. Regardless of other treatment, pigs ate more of the diet supplemented with antimicrobials, utilized the feed with greater efficiency and grew faster than pigs fed the unsupplemented diet ($P<0.001$). There were no interactions between main effects for these parameters. By 8 weeks of age outdoor pigs weaned at 4 weeks of age and fed diets containing antimicrobials were heaviest; pigs reared indoors, weaned at 6 weeks of age and fed unsupplemented diets were lightest (interaction between diet and weaning age $P<0.05$).

**Table 1** Effect of rearing environment, weaning age (4 or 6 weeks of age) and antimicrobial supplementation (-/+) upon ADG, ADI and FCR for 14 days post weaning, and 8 week weight.

| | Indoor | | | | Outdoor | | | | | Probability | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 4 | | 6 | | 4 | | 6 | | | env | age | diet |
| | - | + | - | + | - | + | - | + | *sem* | | | |
| wean wt (kg) | 7.7 | 7.8 | 10.8 | 10.8 | 8.5 | 8.5 | 13 | 13 | *0.27* | *** | *** | ns |
| ADFI (g/d) | 315 | 376 | 409 | 434 | 312 | 364 | 421 | 461 | *23* | ns | *** | *** |
| ADG (g/d) | 194 | 260 | 272 | 327 | 218 | 300 | 301 | 364 | *21.8* | * | *** | *** |
| FCR | 1.62 | 1.44 | 1.55 | 1.36 | 1.43 | 1.19 | 1.46 | 1.34 | *0.05* | *** | ns | *** |
| 8 wk wt (kg) | 15.2 | 17.6 | 14.3 | 15.4 | 16.6 | 19.1 | 17.5 | 18.4 | *0.47* | *** | * | *** |

Key: * $P<0.05$, *** $P<0.001$

**Conclusion.** The addition of antimicrobials to post weaning diets benefited all pigs regardless of rearing environment or weaning age indicating that outdoor rearing and weaning at 6 weeks of age cannot fully compensate for antimicrobial dietary supplementation. However the 6 week weaned, outdoor-reared pig produced the best performance in the absence of antimicrobials. When antimicrobials were included in the diet or when pigs were reared indoors, 4 week weaning produced heavier pigs at 8 weeks of age.

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**076**

# Effect of raw cereal type on digestibility of starch in the weaned piglet

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**Introduction** Starch is of major importance to the young piglet, since by weight it constitutes the largest portion of the diet and is the major energy-yielding component. Usually provided in the form of cereals, the digestibility of starch is fundamental in contributing to the dietary energy requirements of the animal. Two trials were designed in order to examine the effect of variability between raw cereals on the digestibility of starch in the newly-weaned piglet.

**Materials and methods** Piglets (n = 40 per trial) weaned at 28 days of age were individually housed and fed *ad-libitum* one of four diets differing only in raw cereal type. Four cereals were examined in each trial with the same batch of raw soft wheat common to both studies. Cereals were ground through a 1.5 mm screen and incorporated into diets at a rate of 580 g/kg containing no antibiotic growth promoters. Animals used in the trials were of identical genotype but were sourced from a different supplier, therefore the studies are not directly comparable. Experimental period was for 14 days, with digesta samples taken at slaughter at 0.25, 0.5 and 0.75 proportionally along the small intestine from the gastric pylorus to the ileocaecal valve and freeze dried immediately on collection. Representative faecal samples (freshly collected and pooled for each pig) were taken over two time periods to allow coefficients of total tract apparent digestibility during days 1-5 (CTTAD 1) and days 9-13 (CTTAD 2) to be calculated. Samples were analysed for acid insoluble ash (inert marker) and coefficients of intestinal apparent digestibility (CIAD) for starch within the small intestine were determined using a total starch assay kit (AOAC method 996.11, AACC method 76.13). Data were subjected to analysis of variance using a fully randomised design (Genstat 7).

**Results** CIAD and CTTAD values with analysis of variance are shown in Table 1. For both trials, coefficients in the proximal region were negligible. In Trial 1, CIAD was significantly affected by region of the small intestine, with coefficients increasing from mid to distal section (P = <0.001). Overall dietary coefficients within the small intestine were highest for rye and lowest for triticale (P = 0.051). No dietary differences were observed in the faecal data but coefficients increased significantly between collection periods (P = 0.044). Data from Trial 2 revealed a similar increase in starch digestion between the two intestinal sites except for pigs fed the maize based diet where a decrease in digestibility from the mid to distal section was found. Overall dietary coefficients between the cereals were not significantly different although analysis of the faecal data from trial 2 revealed a significant effect of dietary cereal (P = <0.001) and an interaction between diet and collection period.

**Table 1** Coefficients of starch digestion in small intestine (CIAD) and over total tract (CTTAD)

| Trial No. | Cereal | CIAD 0.5 region | CIAD 0.75 region | CTTAD 1 | CTTAD 2 |
|---|---|---|---|---|---|
| 1. | Wheat | 0.527 | 0.871 | 0.966 | 0.997 |
| | Barley | 0.394 | 0.876 | 0.979 | 0.995 |
| | Rye | 0.572 | 0.881 | 0.983 | 0.995 |
| | Triticale | 0.420 | 0.758 | 0.976 | 0.992 |
| 2. | Wheat | 0.616 | 0.740 | 0.994 | 0.989 |
| | Naked Oats | 0.630 | 0.738 | 0.990 | 0.996 |
| | Whole Oats | 0.694 | 0.840 | 0.993 | 0.992 |
| | Maize | 0.610 | 0.524 | 0.978 | 0.960 |

ANOVA

| Trial No. | CIAD | | | CTTAD | | |
|---|---|---|---|---|---|---|
| | Factor | s.e.d. | P | Factor | s.e.d. | P |
| 1. | Cereal | 0.047 | 0.051 | Cereal | 0.011 | 0.893 |
| | Region | 0.034 | <0.001 | Collection Period | 0.008 | 0.044 |
| | Cereal*Region | 0.068 | 0.310 | Cereal*Collection Period | 0.016 | 0.829 |
| 2. | Cereal | 0.069 | 0.098 | Cereal | 0.003 | <0.001 |
| | Region | 0.049 | 0.063 | Collection Period | 0.002 | 0.092 |
| | Cereal*Region | 0.097 | 0.202 | Cereal*Collection Period | 0.005 | 0.035 |

**Conclusion** These results demonstrate differences in the digestibility of starch of raw ground cereals within the small intestine of the weaned piglet. Despite high CTTAD values for all animals, some dietary variation was still apparent in the faecal data. The difference between coefficients at the 0.75 intestinal region and the total tract indicate that fermentation in the large intestine of unprocessed cereals may be considerable in the young piglet. The decrease in digestibility of starch between the two intestinal regions by pigs on the maize diet is difficult to explain. However, the low digestibility evident at both the 0.75 site and from the CTTAD values over both collection periods, suggest the maize diet was the least well-digested cereal throughout the 14 day period.

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# The effect of protein level and diet quality on the performance of newly weaned pigs on a large scale facility

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**Introduction** The prophylactic use of in-feed antimicrobial growth promoters (AGP) in weaner pig diets has been banned within the EU and emphasis must therefore be placed on the development of effective nutritional strategies for disease prevention. Manipulating dietary protein supply (Wellock et al., 2006) and the heat treatment of cereals (Doucet et al., 2006) have been shown to improve gut health, decrease the risk of enteric disease and minimise the post-weaning growth check under experimental conditions, by decreasing substrate availability to potential pathogens, such as enterotoxigenic *E. coli* (ETEC). The aim was to investigate the combined effects of protein level and diet quality on the health and performance of weaned pigs in the absence of AGP and therapeutic levels of ZnO and $CuSO_4$ in a large-scale facility.

**Materials and methods** A 2 x 2 factorial design experiment compared dietary crude protein level (H, 230 g CP/kg vs L, 170 g CP/kg) and diet quality (h, vs l) over five experimental rounds. 400 (JSR Gold x Hampshire) pigs of mixed sex were weaned at 29.4 ± 3.09 days (± S.D.) of age and 9.9 ± 1.62 kg body weight, balanced for initial weight and litter and offered *ad libitum* access to one of the four dietary treatments for 14 days post weaning. The high quality diets contained cooked cereals (wheat, dehulled oats and maize) and animal protein sources (fish meal and dried skimmed milk powder), whereas the low quality diets contained raw cereals (wheat, dehulled oats and maize) and plant protein sources (soya bean meal and full fat soya). All diets were formulated to contain 16.0 MJ DE/kg and were balanced for essential amino acid composition as a proportion of total protein, sodium and lactose content. From day 14 pigs were fed standard commercial diets until the end of the weaner phase at approximately 30 kg body weight (days 14+). Pigs were housed in groups of 7 or 8 whilst on trial. Individual weight gain, pen feed intake and faecal score (FS; assessed on a 4 point scale, where 1 = firm and 4 = watery) were recorded. Fresh faecal samples were collected on day 11 post weaning for culture and enumeration of lactobacilli and coliform populations and to assess ETEC shedding. Data were analysed by ANOVA with pen as the experimental unit, experimental round as a fixed effect and mean pig weaning weight as a covariate.

**Results** Table 1 shows the effect of protein level and diet quality on pig health and performance. Pigs fed high protein and high quality diets gained more (ADG) than those on the low protein and low quality diets respectively over the 0-14 day period. There was no effect of CP level or quality from day 14 to the end of the weaner phase (days 14+). Pigs fed high quality diets ate (ADFI) more than those on the low quality diets over the 0-14 day period. There were no effects of protein level or quality on feed conversion ratio (FCR) or FS, with few pigs displaying diarrhoea. Pigs fed high quality diets shed fewer ETEC and had a higher faecal lactobacilli to coliform ratio than those fed the low quality diets. There were no significant CP level x diet quality interactions.

**Table 1** The effect of crude protein level (H vs L) and diet quality (h vs l) on pig performance, ETEC shedding and lactobacilli to coliform ratio throughout the trial period.

| | Hh | Hl | Lh | Ll | SED | Response |
|---|---|---|---|---|---|---|
| ADG days 0-14 (g/d) | 251 | 212 | 212 | 189 | 22.2 | P*, D* |
| ADG days 14+ (g/d) | 580 | 571 | 573 | 574 | 25.2 | |
| ADFI days 0-14 (g/d) | 346 | 306 | 347 | 313 | 12.8 | D*** |
| ADFI days 14+ (g/d) | 894 | 880 | 890 | 897 | 28.4 | |
| FCR days 0-14 | 1.48 | 1.70 | 1.79 | 1.89 | 0.199 | |
| FCR days 14+ | 1.56 | 1.55 | 1.57 | 1.58 | 0.077 | |
| Faecal Score days 0-14 | 1.97 | 2.06 | 1.95 | 2.01 | 0.043 | |
| ETEC ($log_{10}$ cfu/g) | 4.57 | 5.43 | 4.78 | 6.82 | 0.867 | D* |
| Lactobacilli to Coliform ratio | 1.33 | 1.24 | 1.35 | 1.17 | 0.058 | D** |

Main effects * P < 0.05, **, P < 0.01, *** P<0.001; where P = Protein level and D = Diet quality

**Conclusion** Under the conditions of the trail, pigs fed the high quality diets had improved gut health compared to those fed low quality diets, although all pigs on the trial remained healthy with few cases of diarrhoea. Pigs fed the high protein and high quality diets gained more than those fed the low protein and low quality diets respectively over the 14 day post-weaning period. There was no difference in pig performance from day 14 to the end of the weaner phase suggesting the absence of any compensatory growth. Animals will continue to be monitored until slaughter. Life time performance and a full economic analysis will be calculated.

**Acknowledgements** This research was financially supported by ABNA Ltd, Frank Wright Ltd, Home-Grown Cereals Authority, Meat and Livestock Commission/British Pig Executive, Primary Diets Ltd and Provimi Ltd with match funding from Defra, through the Sustainable Livestock Production LINK programme.

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# Creep feed composition did not affect feed intake, growth rate or gut microflora in weaned pigs

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**Introduction** Although dietary composition for weaned pigs can be altered to promote a beneficial microflora which resists proliferation of pathogenic bacteria, this concept has yet to be applied to the pre-weaning phase. Commercial creep feeds typically are highly digestible and based on milk products and cooked cereals which, in the presence of lactase enzyme activity in the suckling piglet, promote relatively limited microbial fermentation in the gut. The gut flora therefore remains relatively immature and less able to deal with the challenges of weaning and transition to a non-milk diet. Recent studies in weaned piglets have shown how dietary ingredients such as inulin can enhance the proportion of beneficial bacteria in the gut (Wellock *et al.*, 2006), and thus may provide a more stable gut pH and microflora over the weaning transition. Similarly, addition of sodium butyrate to the diet can enhance gut architecture (Miller and Slade, 2006), which could promote development of a more mature gut at weaning if fed prior to this time. The aim of this experiment was to test whether manipulations which have had beneficial effects on enteric conditions in weaned pigs can be used prior to weaning, to promote more favourable gut conditions and thereby reduce the feed intake deficit and growth check associated with the weaning process.

**Materials and methods** A randomised block design was used with 4 treatments and 12 replicate litters, allocated at 7 days of age. Treatments were: Control (C), a high digestibility milk creep (230 g CP, 18 g fibre, 160 g total lactose/kg); Inulin (I), as for C with the addition of inulin at 50g/kg (as Raftifeed); Butyrate (B), as for C with the addition of 3 g/kg esterified butyric acid; Milk (M), no pelleted creep feed offered but instead liquid milk replacer. Diets and water were offered *ad libitum* from 7 days of age until weaning. To promote higher creep feed intake during lactation, separation of the litter from the dam at sow feeding times was used, with the litter enclosed within their own creep area at the side of the farrowing pen (1h/twice per day in week 2, increased to 1.5h in week 3 and 2h in week 4). The experimental creep diet was offered *ad libitum* for 3 days after weaning (pigs previously on the liquid milk treatment received C creep), after which a standard starter diet was introduced gradually to all treatments. Feed intake and piglet weight were taken at weekly intervals until 28 days after weaning. Gut health was monitored by taking faecal samples (3 pigs/replicate litter) at -1, 3, 6 and 17 days after weaning for culture and enumeration of lactobacilli and coliform bacteria populations and the ratio between these two (L:C). Data were subjected to ANOVA, with litter as the experimental unit and replicate as a blocking factor.

**Results** Treatment had no significant effect on overall creep intake prior to weaning, weaning weight or weight gain. Feed intake in week 2 was however higher in the liquid milk treatment. There were no significant treatment effects on post-weaning feed intake, performance, bacteria populations or L:C ratios (an indicator of gut health).

**Table 1** Effect of creep feed composition on mean piglet liveweight, feed intake, growth rate and FCR values

| Parameter | Control | Inulin | Butyrate | Milk[1] | S.E.M. | Significance |
|---|---|---|---|---|---|---|
| Suckling phase | | | | | | |
| Creep intake week 2 (g/day) | 3 [a] | 2 [a] | 3 [a] | 12 [b] | 0.9 | *** |
| Creep intake week 3 (g/day) | 21 | 12 | 15 | 22 | 3.1 | P=0.068 |
| Creep intake week 4 (g/day) | 64 | 36 | 60 | 39 | 8.3 | * |
| Mean creep intake (g/day) | 30 | 16 | 27 | 25 | 3.7 | P=0.074 |
| Weight gain on sow (g/day) | 222 | 215 | 230 | 216 | 8.4 | ns |
| Weaning wt.(kg) | 8.1 | 7.7 | 8.1 | 7.7 | 0.26 | ns |
| Weaner phase (4 weeks) | | | | | | |
| Mean feed intake (g/day) | 535 | 490 | 532 | 475 | 28.2 | ns |
| Weight gain (g/day) | 395 | 347 | 372 | 346 | 20.1 | ns |
| FCR | 1.26 | 1.27 | 1.26 | 1.25 | 0.02 | ns |
| Weight 28 day post wean (kg) | 19.5 | 17.9 | 19.0 | 17.4 | 0.75 | ns |
| $Log_{10}$ L:C population (day 3) | 1.20 | 1.07 | 1.12 | 1.05 | 0.042 | P=0.066 |
| $Log_{10}$ L:C population (day 6) | 1.16 | 1.12 | 1.13 | 1.09 | 0.039 | ns |

[1] estimated from volume of milk replacer consumed and composition of 150g powder/litre water
[ab] Means followed by different superscripts are significantly different at P<0.001

**Conclusions** Although data on intestinal morphology are not yet available, it appears that the creep diet manipulations investigated here did not deliver any added benefits in post weaning feed intake, performance or microbial indicators of gut health beyond that provided by a high quality, commercial-specification creep.

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# The effect of base particle size in complementary feedingstuffs using early maillard reaction products on the performance of post weaning piglets

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**Introduction** The animal feed industry produces various complementary feedstuffs with high levels of crude protein (CP) and synthetic amino acids designed to provide optimum nutrition to the post weaned pig. The use of synthetic amino acids has both advantages and disadvantages and other ways of delivering amino acids are of interest to the feed industry. One possible way of delivering amino acids is through the Maillard reaction; this is the chemical catalysis of the amine group of an amino acid to the carboxyl group of a sugar giving Maillard reaction products (MRP). These molecules occur during cooking, but have been shown to have a variety of other applications (Namiki, 1996) In this study, lysine (Lys), methionine (Met) and threonine (Thr) where chemically reacted with sugar molecules to give *in vitro* early Maillard Reaction Products (MRP). The aim of this study was to assess the effect of a solution of these MRPs with particle sizes of cereal base in a complementary feedingstuff (Matan XL, Devenish Nutrition) on overall diet performance.

**Materials and methods** Four complementary feedingstuffs were formulated and pelleted using MRP solution and differing particle sizes of maize (ground to pass through a screen of size either 2, 3, 5, or 8 mm) at standard concentrations, giving four separate treatments. Each treatment was then incorporated into separate two stage starter diets at 50.0 g/kg, giving eight diets in total. All diets were balanced for protein content (19.2 and 20.1 g/100g), digestible energy (DE)(15.9 and 15.5 MJ/kg), Lys (1.52 and 1.37 g/kg), Met (0.48 and 0.44 g/kg) and Thr (0.98 and 0.89 g/kg), respectively. The performance trial utilised 96 post-weaning piglets over a 27-day period, in pens each containing 4 pigs, giving 24 pigs and 6 replicates per treatment. All pens were balanced for weight, sex and litter. The diets were offered *ad libitum* as appropriate. Average daily intake (ADI, g/day), average daily gain (ADG, g/day), and feed conversion ratio (FCR) were determined and results analysed using analysis of variance (Genstat v8.0), with piglet weaned weight used as a covariate.

**Results** The particle size of the cereal base used in the complementary feedingstuff significantly affected many factors relating to piglet performance (Table 1). The smaller the maize particle size, the greater the difference in the final mean piglet weight (Figure 1). This pattern is reflected in very significant differences in ADI and ADG for the smaller particle bases (2 and 3 mm) compared to that of the larger particle sizes (5 and 8mm). The complementary feedingstuff formed when the 2 mm ground maize is formulated with the MRP solution gives the greatest significant increases in ADI and ADG in comparison to all other grind sizes.

**Table 1** The effect of particle size of maize base (in complementary feedingstuff) on piglet performance

| Maize Particle Size | | 2 mm | 3 mm | 5 mm | 8 mm | Sed | P |
|---|---|---|---|---|---|---|---|
| Wt (kg) | day 1 | 6.90 | 6.90 | 6.90 | 6.90 | NA | NA |
| | 27 days | 15.80[a] | 15.59[a] | 15.50[a] | 14.26[b] | 0.209 | 0.043 |
| ADI (g/d) | | 392.7[c] | 376.8[b] | 376.6[b] | 343.8[a] | 3.98 | <0.001 |
| ADG (g/d) | | 334.1[c] | 311.7[b] | 317.7[bc] | 271.6[a] | 6.61 | 0.002 |
| FCR | | 1.175 | 1.209 | 1.185 | 1.355 | 0.064 | NS |

[a, b and c] values with different superscripts are significantly different ($p<0.05$)

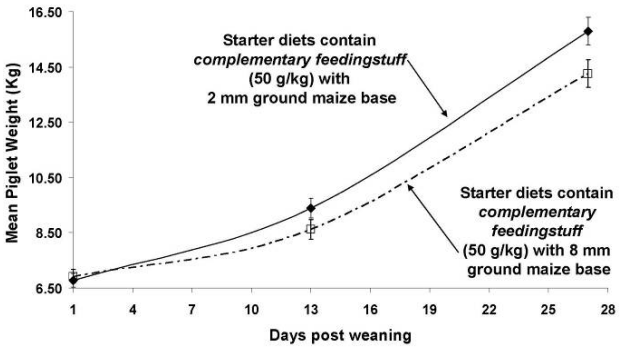


**Figure 1** Pig growth response to 2 and 8 mm maize particle size (in complementary feedingstuff)

**Conclusions** The use of *in vitro* MRP solution (derived from Lys, Met and Thr reacted with sugars) with the smaller the maize particle size (in the complimentary feedingstuff) the greater the improvement in overall diet ADI, which in turn drives significant improvements in pig ADG and final weight (though not significant). It is speculated that the smallest grind size of maize (greatest surface area) gives the best ADI and ADG through greater physical absorption by the base material (and delivery to the gastrointestinal tract) of the amino acids from the MRP solution.

**Acknowledgements** We wish to thank the Agri-Food and Biosciences Institute (AFBI), Queen's University Belfast and Devenish Nutrition, Belfast.

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# Effect of different creep feeding treatments and group composition on the initiation of piglet feeding behaviour immediately post-weaning

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**Introduction** The post-weaning growth check of the piglet can be attributed to the inability of the animal to eat sufficient food in the initial post weaning period and hence grow at a maximum rate (Pluske *et al.*, 1996). It is essential therefore to identify determinants of individual feed intake characteristics post weaning to improve piglet performance. A palatable creep-diet is often fed to piglets pre-weaning to increase performance and to allow piglets to become familiarised with solid feed sourced from a feeder. Bruininx (2002) *et al.,* demonstrated using single spaced Feed Intake Recording Equipment (FIRE), that creep feed stimulates post-weaning feed intake and gain. In addition, Morgan et al. (2001) investigated feeding behaviour of piglets paired from creep or no creep backgrounds and concluded a learning effect which increased the feeding behaviour of the naïve piglet. The purpose of this experiment, therefore, was to investigate the effect of offering selected litters either creep feed or zero creep (naïve) pre-weaning on their latency to initiate feeding post weaning. It was hypothesised that learning behaviour between piglets may influence feeding initiation and so this experiment would also look at the effect of mixing piglets from the two lactation backgrounds.

**Materials and methods** Thirty-six creep fed piglets (C) and thirty-six naïve (No Creep = NC) piglets (JSR Healthbred) were weaned at 7.9kg ±0.22 and 7.7 kg ±0.22 (±sem) liveweight and 28.0 ±0.32 and 27.0 ±0.32 days of age respectively into 18 flat deck pens (4 pigs per pen). Piglets were allocated to one of two group composition treatments: mix (combination of 2 x C with 2 x NC and vice-versa) or same (either 4 x C or 4 x NC) balancing for liveweight, sex and litter origin. Piglet feeding behaviour was recorded 24hrs a day using a multi-spaced FIRE system (Leeds University Pig INtake System (LUPINS)) in each pen from which piglets were offered *ad-libitum* access to feed (16.45 MJ DE, 1.6 g lysine/kg) in four troughs per pen. Piglets were identified by LUPINS using an individual transponder ear tag. Piglets were weighed at d0 and d8 post weaning. Data were analysed as a 2x2 factorial using the GLM procedures of Minitab 12.2, weaning age was included as a covariate.

**Results** The latency time for piglets to initiate feeding is displayed in Figure 1. Piglets experiencing solid feed pre-weaning had significantly reduced latency to first meal regardless of post-weaning group composition ($p<0.05$). The interaction between treatments was significant ($P<0.05$) with the graph clearly showing that latency in naïve piglets was increased markedly by mixing with experienced (C) piglets ($P<0.05$). Growth rate to eight days post weaning was unaffected by pre or post weaning treatments.

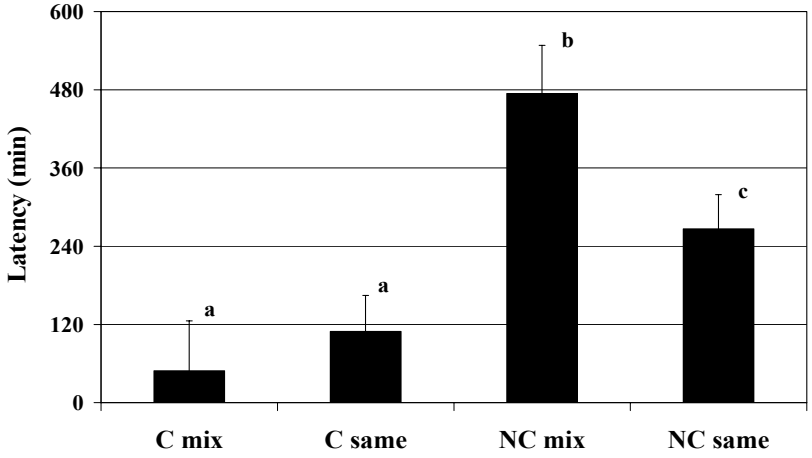


**Figure 1** Effect of pre-weaning creep feed experience and weaning group composition on latency to first post-weaning meal.

**Conclusions** As predicted, the piglets which received creep feed pre-weaning had much better feeding initiation than naïve piglets who had no experience of solid feed. However, the effect of mixing C and NC piglets was unexpected with the NC (naïve) piglets having significantly longer latency times when mixed with C (experienced) piglets. Therefore, the results of this study do not support the hypothesis of positive information transfer between piglets differing in exposure to creep feed pre-weaning.

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**081**

# Effects of housing system and litter grouping on performance and physiological maturity in entire boars and gilts

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**Introduction** Slaughter weight in UK pig production is increasing annually, with an associated increase in age at slaughter. This increases risk of reaching sexual maturity and a reduction in carcass quality and production efficiency. In entire boars, puberty is associated with increased aggression levels and the production of hormones causing carcass taint. Management systems and production practices may affect these risks. Thomas *et al* (1979) found that extensive housing and management systems increased the risk of pigs reaching puberty. In previous Scandinavian research, Fredriksen *et al* (2005) found that maintaining stable litter groups reduced aggression and mounting behaviour, and gave a lower propensity for carcass taint at slaughter. To date, there is a lack of research examining the effects of these factors on physiological development and aggression under UK conditions. This study explored the effect of stable litter grouping and housing system on performance, aggression and physiological maturity in entire males and gilts over the 60kg-130kg weight range.

**Materials and methods** 128 Large White x Landrace pigs (64 boars, 64 gilts) approx. 60kg live weight (LWT), were selected from either stable litter groups (LM) or groups containing litters mixed at weaning (NLM) and allocated to mixed gender pens of eight in extensive straw yard housing (ST) or controlled environment housing with unbedded part-slatted pens (CE) Mean slaughter weight was 125kg LWT. Pigs were fed a commercial pelleted finisher diet *ad-libitum,* with pig live weight and pen feed intake recorded fortnightly from 60kg to 100kg and weekly from 100kg to slaughter. Skin lesion scores were recorded fortnightly and rindside damage post slaughter. For an assessment of maturity, ovary weights and follicle scores or testicle weights were recorded at slaughter. Pen mean values were subject to two-way analysis of variance, with housing system, litter grouping treatments and their interaction as factors, to evaluate effects on performance, physiological maturity and aggression measures.

**Results** Over this heavy weight range, boars showed higher daily live weight gain (DLWG) compared to gilts. There was no effect of housing or litter treatment on DLWG, daily feed intake (DFI) or feed conversion ratio (FCR). ST pigs exhibited a significantly higher back fat thickness compared to CE pigs ($p<0.001$). Age at slaughter was significantly affected by gender ($p<0.001$) and litter treatment ($p=0.011$) but not housing. Mean skin lesion scores were not affected by housing or litter treatment. Boars exhibited significantly greater rindside damage compared with gilts ($p=0.039$). When analysed separately for boars, rindside damage was significantly greater in the NLM treatment, ($p=0.006$). Straw yard housing significantly increased gilt ovary ($P=0.017$) and uterine ($P=0.034$) weights. NLM treatment significantly increased gilt uterine weight ($p=0.027$) and boar testicle weight ($p=0.033$).

**Table 1** Effects of gender (G), housing (H) and litter (L) treatment on performance, physiological maturity and aggression measures

| | Controlled Environment | | | | Straw Yard | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | LM | | NLM | | LM | | NLM | | SE | Sig |
| | B | G | B | G | B | G | B | G | | |
| DFI (kg) | 2.48 | | 2.25 | | 2.42 | | 2.64 | | 0.152 | - |
| FCR | 2.99 | | 2.52 | | 2.61 | | 3.04 | | 0.091 | - |
| DLWG (kg) | 0.87 | 0.79 | 0.96 | 0.84 | 0.96 | 0.88 | 0.90 | 0.84 | 0.022 | G** |
| Slaughter back fat (mm $P_2$) | 13.5 | 12.8 | 13.7 | 12.8 | 14.9 | 15.9 | 15.7 | 15.9 | 0.58 | H*** |
| Age at slaughter (days) | 191 | 207 | 184 | 189 | 190 | 198 | 191 | 198 | 2.1 | G***,L* |
| Rindside damage score [a] | 1.8 | - | 2.2 | - | 1.8 | - | 2.8 | - | 0.18 | L** |
| Gilt uterus weight (g) [b] | - | 216 | - | 301 | - | 270 | - | 687 | 84.1 | L*, H* |
| Gilt pair ovary weight (g) | - | 7.23 | - | 7.40 | - | 10.13 | - | 13.58 | 1.319 | H* |
| Testicle Weight (g) [b] | 600 | - | 625 | - | 512 | - | 663 | - | 27.5 | L* |

[a] boar only data, [b] slaughter live weight as covariate

**Conclusion** Whilst there was no effect of housing on growth rates, pigs reared in straw yard housing were significantly fatter. The physiological response to housing appeared to be gender specific with gilts reared in straw yards being significantly more mature at slaughter. The response to mixed litter grouping was more apparent in the boars, with NLM boars being more physiologically mature at slaughter and showing increased levels of carcass damage. Blood and tissue samples taken at slaughter are being analysed to quantify risk of carcass taint.

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# The effect of crude protein and lysine level in diets for finishing pigs on nutrient digestibility and nitrogen balance

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**Introduction** Due to the introduction of more stringent environmental controls through the implementation of current and impending environmental legislation, dietary crude protein (CP) levels for finishing pigs are an important area of research. Canh *et al* (1998) reported that nitrogen (N) excretion could be reduced through lowering CP levels and the aim of this study was to investigate the effect of decreasing CP levels of diets containing two levels of lysine (while maintaining the ideal protein balance through amino acid supplementation) on nutrient digestibility and N balance.

**Materials and methods** Ten diets for finishing pigs were formulated to contain decreasing levels of CP (188 to 136g/kg fresh basis) at two levels of available lysine (9 and 8g/kg fresh basis). The diets were formulated to contain the major essential amino acids close to ideal protein as determined by Wang and Fuller (1990). The dietary treatments were offered to a total of 80 LWxLR pigs over eight time periods giving eight replicates/treatment. The average start weight of the pigs was 39.1kg and pigs were housed in metabolism crates, for a period of 14 days (7d prefeed + 7d balance collection). Samples of the diets, faeces and urine were analysed to determine digestibility of dry matter (DM), crude protein (CP), oil, neutral detergent fibre (NDF) and energy. N balance figures were determined (from total collection of faeces and urine) and also calculated from liveweight gain (LWG) measured in a corresponding performance trial using the equation cited by Whittemore *et al* (1988) (N retention = 0.16 * LWG). Diets offered in the performance trial were identical to the diets offered in this trial. The results were analysed by ANOVA to determine differences between means and linear regression contrasts.

**Results** DM, CP and NDF digestibility decreased linearly (0.826 to 0.815, 0.831 to 0.758, and 0.524 to 0.446 respectively) and oil digestibility increased linearly (0.692 to 0.758) with decreasing CP content. N retention and excretion decreased linearly with decreasing CP content. N retention and excretion figures calculated from the LWG measured in the performance trial were lower than those determined through balance collection (on average, 24% difference in N retention) (Table 1). N retention, total N excretion and N excretion per pig place decreased with decreasing CP content. Overall, N excretion per pig place was lower than the figure quoted by DEFRA (2002) (10.5kg) for finishing pigs. There was no significant effect of available lysine level on nutrient digestibility or N balance.

**Table 1** Effect of dietary crude protein on nitrogen balance

| | N Balance (g/d) – as determined (BALANCE – faeces and urine collection) | | | | | N Balance (g/d) – as calculated (0.16 * LWG) | | | |
|---|---|---|---|---|---|---|---|---|---|
| Dietary CP (g/kg) | Ret | Ur Ex | F Ex* | Total Ex | N Ex kg/pig place[#] | Ret | Ur Ex | Total Ex | N Ex kg/pig place[#] |
| 136 | 24.2[a] | 6.6[a] | 7.9[a] | 14.4[a] | 4.73[a] | 22.0 | 8.8 | 16.7 | 5.47 |
| 149 | 26.6[b] | 8.1[b] | 9.6[b] | 17.7[b] | 5.79[b] | 22.1 | 12.6 | 22.2 | 7.27 |
| 162 | 26.9[bc] | 11.0[c] | 9.3[b] | 20.2[c] | 6.63[c] | 21.7 | 16.1 | 25.4 | 8.32 |
| 175 | 28.5[bc] | 13.1[d] | 9.4[b] | 22.2[d] | 7.32[d] | 21.7 | 20.0 | 29.4 | 9.63 |
| 188 | 28.8[c] | 15.3[e] | 9.0[b] | 24.4[e] | 7.89[d] | 21.4 | 22.7 | 31.7 | 10.40 |
| SEM | 0.710 | 0.497 | 0.369 | 0.613 | 0.204 | | | | |
| P | <0.001 | <0.001 | <0.05 | <0.001 | <0.001 | | | | |
| P (Lin) | <0.001 | <0.001 | NS | <0.001 | <0.001 | | | | |

[a,b,c,d] Means without a common superscript are significantly different. Ret = retention, Ur Ex = Urinary excretion. F Ex = Faecal excretion. * N in faeces is not subject to volatilisation, therefore F Ex as determined by BALANCE is applicable to calculated. Total Ex = total N excretion. [#]N Ex kg/pig place was calculated using the assumptions made by DEFRA in calculating N excretion/pig place = 84 days in the finishing stage and 90% occupancy.

**Conclusions** Nutrient digestibility decreased with decreasing dietary CP content, which would tend to increase slurry DM output. N excretion was determined from balance studies and calculated from LWG. The LWG method gave the highest possible value as it included all losses. N excretion determined through balance was underestimated as N was lost during collection as ammonia, through volatilization of urine. However, regardless of how N excretion was determined, lowering CP lowered N excretion, which would ultimately lower N output in the slurry and that lost through volatilization, which is in line with the report by Canh *et al* (1998). Under the conditions of this study, even at the highest level of CP, N excretion/finishing pig place was considerably less than that proposed by DEFRA (2002).

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# The effect of changing diet in an abrupt, phased or free-choice manner on performance of pigs from wean to finish

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**Introduction** In commercial pig production, several diets are offered to pigs dependent on their age and/or weight. A common dietary regime includes at least four changes of diet from wean to finish but there is a lack of information in the literature regarding the optimum method of changing diet. A common practice is to abruptly change from one diet to the next, although an abrupt diet change has been reported to cause a disturbance in the gut flora resulting in diarrhoaea, especially in the post-weaning stage (Meyer *et al* 1974). The aim of the current study was to investigate the effect of changing diet in an abrupt, phased or free-choice manner on the performance of pigs from wean to finish.

**Materials and methods** A total of 480 pigs (Large White x Landrace) were housed in groups of 20 at weaning, over eight time replicates. Each group was balanced for gender and weight with six groups of large (10.5kg), medium (9.2kg), small (7.7kg) and mixed weight (9.2kg) pigs respectively. Two of the pens for each pig weight group were offered dietary changes in an abrupt, phased or free-choice manner giving a total of eight pens on each method of diet change. The dietary regime was as follows: 3kg of Starter 1, 6 kg of Starter 2, Grower diet until 11 weeks of age and then Finisher diet until finish at 152 days. Digestible energy contents of Starter 1, 2, Grower and Finisher diets were 16.1, 15.3, 14.2 and 13.5 MJ/kg respectively. Crude protein contents of Starter 1, 2, Grower and Finisher diets were 224, 197, 200 and 180g/kg respectively. For the abrupt treatment, pigs were not given a period of diet acclimatisation but underwent an immediate change from diet to diet. For the phased treatment, the new diet was introduced gradually five days prior to the change over in increasing proportions mixed with the previous diet (0.2:0.8, 0.4:0.6, 0.6:0.4, 0.8:0.2, 1.0:0.0 new:previous diet). For the free-choice treatment, feeders were divided and the new and previous diet offered simultaneously for five days. Daily liveweight gain (LWG), daily feed intake (FI) and feed conversion ratio (FCR) were determined from weaning to 7 and 10 weeks of age and from 10 weeks of age until finish at 152 days. The results were analyzed by Analysis of Variance (single factor, no blocking) using Genstat 8.

**Results** The method of changing the diet had no significant effect on pig performance at any stage of production except for feed efficiency from 10 weeks of age until finish (Table 1). Pigs undergoing an abrupt change of diet were more efficient than those on the other dietary regimes. There was no interaction between weight of pigs in the pen and method of diet change.

**Table 1** Effect of method of changing diet on pig performance

| | Abrupt change | Phased change | Free-choice | SEM | P |
|---|---|---|---|---|---|
| Wean to 7 weeks | | | | | |
| Average daily gain (g/d) | 340 | 349 | 338 | 7.98 | NS |
| Feed intake (g/d) | 416 | 422 | 405 | 10.9 | NS |
| FCR | 1.24 | 1.22 | 1.21 | 0.024 | NS |
| 7 to 10 weeks | | | | | |
| Average daily gain (g/d) | 710 | 695 | 676 | 23.2 | NS |
| Feed intake (g/d) | 1159 | 1106 | 1097 | 32.7 | NS |
| FCR | 1.63 | 1.60 | 1.63 | 0.034 | NS |
| *10 weeks to finish* | | | | | |
| Average daily gain (g/d) | 848 | 848 | 823 | 16.3 | NS |
| Feed intake (g/d) | 2107 | 2169 | 2110 | 46.1 | NS |
| FCR | 2.49[a] | 2.56[b] | 2.57[b] | 0.022 | <0.05 |

[a,b] Means with different superscripts are significantly different (P<0.05)

**Conclusions** Abruptly changing the diet did not result in a reduction in pig performance in comparison to the other treatments. This suggests that there was no detrimental effect on gut microflora or activity which is in contrast to Lesniewska *et al* (2000) and Meyer *et al* (1974). Furthermore, an abrupt change of diet improved the efficiency of feed use by pigs in the finishing stage (10 weeks to finish), which may be attributed to reduced feed wastage.

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# The digestibility and performance of complementary feedingstuffs using maillard reaction products in the post weaning pig

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**Introduction** The addition of 'high quality' complementary feedingstuffs to the diet of the post weaning pig can positively impact on growth and lean muscle deposition. The Maillard Reaction bonds amino acid and sugar molecules together, and is one of the major pathways in the chemical changes that occur in the cooking process. Cooking of feedingstuffs has been shown to improve the digestibility and nutritive value of a diet (Pickford et al, 1992). In this study, lysine (Lys), methionine (Met) and threonine (Thr) where chemically reacted with sugar molecules to give *in vitro* early Maillard Reaction Products (MRP). The aim of this study was to assess what effect the addition of a solution of these MRPs to a complementary feedingstuff (Matan XL) would have on overall diet digestibility and subsequent piglet performance.

**Materials and methods** Three complementary feedingstuffs treatments were created using an *in vitro* MRPs solution (derived from reacted Lys, Met and Thr and sugars) and either maize, HiPro soya, or wheat bases, all at standard concentrations. Each treatment was then formulated into separate two stage pig starter diets at a concentration of 25 g/kg, giving six diets in total. All treatments were balanced for crude protein content (CP)(20.6 and 20.2 g/100g), digestible energy (DE)(15.7 and 15.2 MJ/kg), Lys (1.52 and 1.37 g/kg), Met (0.48 and 0.44 g/kg), Thr (0.98 and 0.89 g/kg), with titanium dioxide ($TiO_2$) added as an indigestible marker. Diets were offered *ad libitum*. The digestibility trial utilised 18 post-weaning piglets, over a 14-day period in individual metabolism cages, with 6 piglets per treatment. Faeces of the individual animals were collected from days 7 to 14. At slaughter, empty carcass weight was measured, ileal contents collected, freeze dried, analysis performed and digestibility parameters calculated. Average daily intake (ADI, g/d), average daily gain (ADG, d/g), and feed conversion ratio (FCR) were determined. Results were analysed using analysis of variance (Genstat v8.0), with the weight of piglets at weaning used as a covariate factor. Diets and faeces were analysed for dry matter (DM), neutral detergent fibre (NDF), crude protein (CP), and oil B. Ileal contents were analysed for total starch (TS) and $TiO_2$ to calculate ileal digestibility.

**Results** The type of cereal base used in conjugation with the MRP solution significantly affected final piglet weight, ADI and ADG, but not FCR (Table 1). Significant differences were also observed in measured empty carcass weight at the end of the trial, with the maize-based feedingstuffs giving the heaviest carcasses (Table 1). However, no significant differences in digestibility were observed between any of the laboratory analysed parameters (Table 2).

**Table 1** The effect of complementary feedingstuffs base on piglet performance

| | | Maize | Soya HiPro | Wheat | Sed | P |
|---|---|---|---|---|---|---|
| Wt (kg) | Day 1 | 9.60 | 9.60 | 9.60 | NA | NA |
| | 14 days | 14.62$^{c}$ | 12.97$^{a}$ | 14.10$^{b}$ | 0.47 | 0.050 |
| ADI (g/d) | 0 – 14 d | 359.0$^{b}$ | 261.0$^{a}$ | 334.0$^{b}$ | 21.7 | 0.008 |
| ADG (g/d) | 0 – 14 d | 358.0$^{b}$ | 241.0$^{a}$ | 321.0$^{a}$ | 33.7 | 0.050 |
| FCR | 0 – 14 d | 1.003 | 1.083 | 1.040 | 0.138 | NS |
| Carcass wt (kg) | day 14 | 11.80$^{c}$ | 10.42$^{a}$ | 11.41$^{b}$ | 0.38 | 0.038 |

$^{a, b \& c}$ values with different superscripts are significantly different ($p<0.05$)

**Table 2** The effect of complementary feedingstuffs base on diet digestibility

| | | Maize | Soya HiPro | Wheat | Sed | P |
|---|---|---|---|---|---|---|
| Faecal Digestibility | DM | 0.876 | 0.888 | 0.877 | 0.025 | NS |
| Co-efficient | NDF | 0.662 | 0.739 | 0.682 | 0.022 | NS |
| | CP | 0.831 | 0.842 | 0.824 | 0.017 | NS |
| | Oil B | 0.816 | 0.820 | 0.767 | 0.032 | NS |
| Ileal Digestibility | | | | | | |
| Co-efficient (Days 7-14) | TS | 0.894 | 0.905 | 0.894 | 0.025 | NS |

**Conclusions** The formation of MRPs *in vivo* in cooking is well documented as well as their impact on the digestibility and nutritive value (Bjorck et al. 1984). The employment of MRPs in complementary feedingstuffs with a maize base, improves post weaning piglet performance and muscle deposition (assessed by empty carcass weight) over that of other commercial bases employed, when included at 25 g/kg into two stage pig starter diets. However, the mechanism by which the MRPs solution exerts these effects in the post weaning period is not through changes in feedingstuff digestibility, or by changes in the FCR of the diet.

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# AGEWEAN – The effect of weaning age on sow performance over four parities

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**Introduction** Weaning pigs from the sow at an older age, when their digestive systems are more mature, has been suggested as an approach to reduce the potentially negative effect of the in-feed antibiotic growth promoter (AGP) ban on the national pig herd. Whilst this approach has been shown to improve feed intake and piglet growth rate during the early postweaning period (Edge et al. 2006) it is also important to consider how changes in weaning age may influence sow productivity and longevity in the herd. The AGEWEAN programme of research followed 570 gilts whose piglets were weaned at either 4, 6 or 8 weeks of age through four successive parities; reproductive performance, litter data and the timing and reasons for any sow being culled from the herd were recorded.

**Materials and methods** The research was carried out at 6 separate experimental sites, chosen to represent a range of diverse geographical locations and production systems within the UK. Two sites were used to provide an outdoor lactation environment (total of 100 sows per weaning age treatment) and four sites to provide the indoor environment (total of 90 sows per treatment). The three weaning age treatments were 4 weeks (weaned at between 21 and 28 days of age), 6 weeks (35-42 days of age) and 8 weeks (49 to 56 days of age). On any given site, contemporary blocks of gilts were introduced at the point of farrowing and were followed through four consecutive parities where measurements taken included sow body weight and body condition, piglet performance and sow and piglet feed intake. The effects of weaning age were analysed (in Genstat) using ordinal regression (for the number of completed parities), generalized linear mixed models (for frequencies such as numbers born) or linear mixed models: the mixed models included fixed effects for weaning age and random effects for site and site by weaning age interactions.

**Results** The effects of weaning age were similar in indoor and outdoor herds when analysed separately, and data were then combined for presentation (see Table 1). There were no significant effects of weaning age on the total lifetime number or weight of piglets born, or their mean birth weights. Similarly, there was no effect of weaning age on the total number of pigs weaned, although weaning weight increased as would be expected. There was no significant effect of weaning age on the number of sows removed from trial before completing 4 parities, or on the reasons for removal. With regards to the longevity of the sows, there was also no significant effect of weaning age on the mean number of parities that sows completed. Whilst the wean-farrow interval was longer in 4 week sows, this did little to offset the longer lactation length on the other treatments. There was thus a significant effect of weaning age on the numbers of piglets produced per sow/day on trial, which approximated to the 4 week weaned sows producing 4 more weaned piglets/sow/year than the 8 week weaned sows.

**Table 1** Lifetime productivity of sows weaned at 4, 6 or 8 weeks post partum over 4 successive parities

| | 4 week | 6 week | 8 week | sed | sig |
|---|---|---|---|---|---|
| Number of completed parities | 3.59 | 3.54 | 3.41 | ~ | ns |
| Wean-Farrow interval (days) | 131.69 | 126.07 | 129.34 | 1.83 | ** |
| Lifetime: total pigs born alive | 40.9 | 40.6 | 39.5 | ~ | ns |
| Lifetime: total weight of pigs born (kg) | 65.2 | 64.2 | 63.6 | 2.8 | ns |
| Mean Pig Birthwt (kg) | 1.53 | 1.52 | 1.52 | 0.03 | ns |
| | | | | | |
| Lifetime: total pigs weaned | 33.7 | 33.1 | 30.7 | ~ | P<0.1 |
| Lifetime: total weight of pigs weaned (kg) | 266 | 385 | 524 | 36.0 | *** |
| Mean pig weight at weaning (kg) | 7.89 | 11.57 | 16.63 | 0.78 | *** |
| Pigs weaned/day on trial[#] | 0.076 | 0.071 | 0.065 | 0.003 | *** |

~ Comparisons were made on a log or logit scale and the estimated mean values back-transformed; hence no sed is shown.
# Days on trial calculated from farrowing date of parity one to weaning date of parity four (or the date at which the sow was removed from trial).

**Conclusion**
This study found no significant effects of increasing weaning age on the lifetime productivity of the sows as measured by total numbers of piglets born alive and weaned or sow longevity within the herd. However there were significant effects of weaning age on the number of piglets produced/day on trial which, in a commercial production system, would lead to a lower productive output per sow/year on the later weaning treatments. Further economic analyses need to be carried out to investigate the trade off between reduced piglet output per sow/year and improved piglet performance during the early postweaning period on the commercial viability of later weaning.

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# The effect of dietary short chain fructo-oligosaccharide inclusion on performance and the digesta concentration of skatole in heavy weight finishing pigs

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**Introduction** As nutrient density requirement decreases with increasing age, finishing pig diets may oversupply nutrients. This can result in excess protein, including the amino acid tryptophan, reaching the large intestine where it can be metabolised by the microbial flora providing a substrate for bacterial skatole production. Skatole can give rise to carcass taint. The use of non-starch polysaccharides (NSP), has been proposed to reduce the levels of production and absorption of skatole. One type of NSP, short chain fructo-oligosaccharides (scFOS), has shown to influence the microbial population and reduce skatole production *in vivo* (Xu *et al*. 2002). It is thought the reduction in skatole production is due to an increased requirement for amino acids for bacterial cell protein synthesis, combined with changed microbial biota and pH which shifts the microbial metabolism of tryptophan toward indole production. This study explored the effect of a dietary inclusion of scFOS on the production of skatole and performance of a commercial UK genotype taken to heavier slaughter weight.

**Materials and methods** 112 Large White x Landrace finishing pigs of 57 $\pm$ 1.15 kg live weight were blocked by age and weight and allocated to give four replicate pens of each gender (entire males or females) per treatment. Control pigs were fed a commercial pelleted finisher diet (18% CP, 13%DE, 1.1% Lysine) *ad-libitum.* The scFOS treatment pigs were fed the same control diet with a 0.2% addition of a scFOS preparation (2kg/ton), containing kestose, nystose and fructosyl nystose. Pen feed intake was calculated weekly. Pigs were weighed weekly to calculate daily live weight gain (DLWG) with back fat at the P2 position measured ultrasonically. Faecal samples were taken at each weighing, and colon content samples were taken at slaughter. Data were analysed using two-way analysis of variance with gender, dietary treatment and their interaction as factors. The statistical unit was the pen for performance data and the individual pig for skatole and indole concentrations.

**Results** Mean slaughter weight was 124kg live weight. Boars had higher daily live weight gain ($p<0.001$) and a significantly improved feed conversion ratio (FCR, $p=0.007$) compared with gilts. Faecal indole concentration increased over time ($p<0.001$), although there was no effect of time on faecal skatole concentration. There was no overall effect of gender on faecal skatole and indole concentrations. Although boars exhibited a significantly higher faecal skatole at slaughter ($p=0.04$), colonic skatole and indole concentration at slaughter did not differ. The dietary treatment showed no overall effect on faecal skatole or indole concentrations, despite a significantly higher faecal skatole concentration in scFOS fed pigs at the end of week 1 ($p=0.03$). There was a significantly increased proximal colon indole concentration at slaughter in the scFOS fed pigs. In the distal colon, skatole concentration was greater than in the proximal colon, but with no gender or dietary treatment difference.

**Table 1** Effects and interactions of gender and diet on performance parameters and indolic compounds

| | Control | | scFOS | | | Sig. | | |
|---|---|---|---|---|---|---|---|---|
| | boars | gilts | boars | gilts | SEM | Gender | Diet | Interaction |
| DFI (kg)[a] | 2.76 | 2.81 | 2.85 | 2.68 | 0.059 | NS | NS | NS |
| DLWG (kg) [a] | 0.90 | 0.82 | 0.97 | 0.82 | 0.020 | ** | NS | NS |
| FCR [a] | 3.1 | 3.4 | 2.9 | 3.3 | 0.01 | ** | NS | NS |
| Slaughter back fat (P2 mm)[a] | 13.1 | 14.8 | 13.7 | 13.3 | 0.37 | NS | NS | * |
| Week 1 faecal skatole (µg/g) | 13.30 | 16.40 | 19.22 | 19.38 | 1.984 | NS | * | NS |
| Week 1 faecal indole (µg/g) | 4.50 | 4.77 | 4.44 | 6.14 | 0.754 | NS | NS | NS |
| Slaughter faecal skatole (µg/g) | 15.11 | 13.88 | 17.36 | 11.18 | 1.742 | * | NS | NS |
| Slaughter faecal indole (µg/g) | 10.27 | 8.73 | 8.06 | 9.20 | 1.038 | NS | NS | NS |
| Proximal colon skatole (µg/g) | 6.12 | 5.39 | 4.06 | 4.57 | 1.143 | NS | NS | NS |
| Proximal colon indole (µg/g) | 6.44 | 6.47 | 7.87 | 9.29 | 1.248 | NS | * | NS |
| Distal colon skatole (µg/g) | 8.77 | 7.71 | 9.96 | 8.78 | 1.269 | NS | NS | NS |
| Distal colon indole (µg/g) | 6.28 | 5.29 | 5.48 | 6.52 | 0.680 | NS | NS | NS |

[a] pen mean values

**Conclusion** Boars were more efficient than gilts at this heavier weight range, but the level of back fat surprisingly exhibited no difference between the sexes. Faecal indole concentration increased with age, but faecal skatole did not reflect this increase and did not show the hypothesised corresponding decrease. An additional 0.2% dietary inclusion of a scFOS product had no effect on performance and no consistent significant effect on skatole or indole concentrations in faeces or in the colon digesta at slaughter. The consequences for carcass taint from these intestinal levels of indolic compounds are being examined, but our results suggest no beneficial reduction at this dietary inclusion level of the scFOS product.

**Acknowledgments** We thank the MLC for a studentship for ELRS and the staff at Cockle Park farm for their technical assistance.

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**087**

# The effect of low protein diets on fat deposition in pigs

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**Introduction** A main aim of modern pig production is to reduce nitrogen excretion to the environment, capturing more of the dietary protein in saleable meat. One way to achieve this is to reduce dietary protein level but this is likely to increase fat deposition, especially in late-developing fat depots. Meat quality will also be affected and these effects will all be influenced by breed type. This study compared three nutritional strategies differing in dietary protein provision in terms of their effects on growth and fat deposition.

**Materials and methods** Forty eight entire male pigs (24 0.75 Large White, LW and 24 0.75 Duroc, D) were reared from 40 to 120 kg live weight on one of three nutritional strategies (Table 1). When protein and lysine were reduced the amino acid profile was maintained, relative to lysine. The pigs were reared in diet and breed groups and fed *ad libitum* from hoppers. At 120 kg they were transported to the University of Bristol where all internal organs and fat depots were removed and carcasses jointed. Joints were dissected into lean, bone and fat. Samples of subcutaneous fat from the hindloin were assessed for fatty acid content and composition.

**Table 1** Nutritional Strategies

| | Baseline | | | Good practice (GP) | | | Low N (LN) | | |
|---|---|---|---|---|---|---|---|---|---|
| Live wt range | [a]DE | [b]CP | [c]L | 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 | 07 |

[a]MJ/kg [b]g crude protein/kg [c]g lysine/kg

**Results** The growth rates and FCRs of pigs on the low protein strategies (GP and LN) were similar to Baseline, despite a lower protein intake (Table 2). GP and LN tended to have fatter carcasses (NS) and marbling fat was increased in LW but not in D on GP and LN. D had more marbling fat and a lower proportion of 18:2, the main polyunsaturated fatty acid in marbling fat.

**Table 2** Growth and composition and significance of breed (B) and nutritional strategy (N) effects

| | Baseline | | Good practice | | Low N | | Sig | |
|---|---|---|---|---|---|---|---|---|
| | LW | D | LW | D | LW | D | B | N |
| Growth (pen basis) | | | | | | | | |
| Growth rate (g/d) | 964 | 932 | 1070 | 992 | 1012 | 1008 | | |
| FCR | 3.00 | 3.01 | 2.69 | 2.88 | 2.91 | 2.87 | | |
| Feed protein intake (kg) | 42.7 | 42.0 | 33.9 | 32.1 | 34.3 | 35.6 | | |
| Composition | | | | | | | | |
| $P_2$ (mm) | 16.0 | 18.0 | 17.0 | 21.0 | 19.0 | 20.0 | NS | NS |
| Fat[a] | 170 | 170 | 180 | 200 | 180 | 200 | NS | NS |
| Marbling fat[b] | 9.8 | 24.0 | 14.6 | 24.9 | 13.8 | 24.1 | *** | NS |
| 18:2[c] | 170 | 123 | 150 | 110 | 150 | 107 | *** | NS |

[a]g/kg carcass side, [b]total fatty acids in loin muscle (g/kg), [c]g/kg total fatty acids

**Conclusions** Reducing protein intake in the GP and LN groups had few negative effects on growth traits but tended to increase fat deposition. Within muscle the effect of low protein diets was to increase marbling fat in LW but not D which had more marbling fat.

**Acknowledgements** We acknowledge funding from Defra.

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# Development of a model to investigate the interaction between host nutrition, genotype and gastro-intestinal parasitism in lambs

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**Introduction** Gastrointestinal parasitism in lambs is usually manifested as a sub-clinical infection and causes significant losses in performance. Its control through the use of chemoprophylaxis is no longer sustainable due to the development of parasitic resistance to anthelminthics, but also due to environmental and consumer concerns. There is thus an urgent need to develop alternative, sustainable methods of controlling gastrointestinal parasitism. These include dietary supplementation of host with protein. The aim here is to develop a mathematical simulation model for the description of the joint effects of nutrition and host performance genotype on the outcome of sub-clinical challenge by gastrointestinal parasitism for growing lambs.

**Materials and methods** The growth of the animals was described using a Gompertz growth function (Wellock *et al*. 2004). The challenge with gastrointestinal parasites was assumed to affect: (i) the protein requirements of the animal (ii) utilization of protein and (iii) its food intake (anorexia). The impact of parasitism was described through the protein loss caused by parasitism and the response through immunity of the host for the minimisation of the protein loss. The potential protein loss is a function of both larval intake and worm burden. In line with previous published models (Bishop and Stear, 1997) the impact of host immunity was modelled through three host controlled traits: (i) establishment of incoming larvae (ii) fecundity of adult female worms (iii) mortality of adult worms. Development of immunity was assumed to be a function of cumulative larval intake. The expression of immunity was a function of protein availability. When protein is scarce, maintenance requirements were assumed to be satisfied first. Protein available above maintenance is allocated to immunity and production traits proportionally to their requirements. Parasitic challenge will lead to increased protein demands for covering the additional immunity and maintenance requirements. The effect of parasitism on food intake, i.e. anorexia, was modelled as a function of worm burden.

Two genotypes with respect to growth were modelled: (i) fast growing similar to Suffolk and (ii) slow growing similar to Scottish Blackface. Three nutritional regimes were simulated: (i) *ad-libitum* on good quality grass (12.6 MJ/kg DM ME and 0.19 kg CP/kg DM) (ii) *ad-libitum* on poor quality grass (7.5 MJ/kg DM ME and 0.097 kg CP/ kg DM) and (iii) restricted on poor quality grass. Two levels of challenge were used: (i) control (no challenge) (ii) high challenge (6000 L3 *Teladorsagia* per day).

**Results** Predicted food intakes and egg counts (FEC) are shown in Figures 1 and 2. Both genotypes showed similar patterns of food intake under different challenges and therefore only the food intake of the fast growing genotype is shown. The fast growing genotype had lower FEC on both good and poor quality grass, *ad-libitum*. This was due to the higher volume of faeces rather than improved immunity. On the other hand when given access to poor quality grass, restricted, the FEC of the slow growing genotype were lower, as a result of the partition of higher proportion of protein towards immunity.

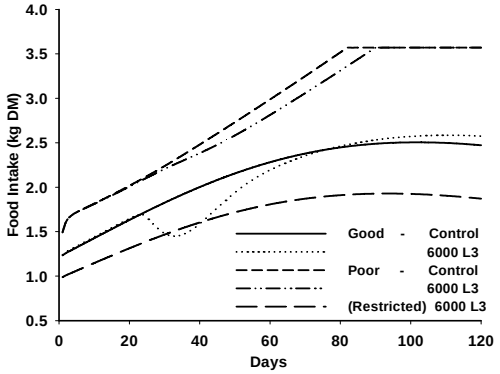


**Figure 1** Predicted food intake of the fast growing genotype, for different levels of challenge and nutritional regimes.

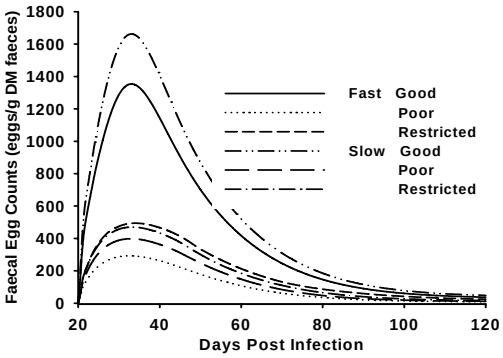


**Figure 2** Predicted faecal egg counts for two lamb genotypes (fast and slow growing) under different nutritional regimes.

**Conclusions** The current model describes the interaction of gastrointestinal parasitism with nutrition for lambs with different expected growth rates. It therefore provides the means for exploring the effect of nutrition and genotype on the performance of parasitized lambs kept in different environments. Expansion of the model to population level will provide the opportunity to investigate the effect of nutrition, host genotype and parasitism on predicted genetic parameters for growth and immunity traits.

**Acknowledgements** Financial support of BBSRC and SEERAD is gratefully acknowledged.

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089

# In silico investigation of the effect of nutrition on the estimates of genetic parameters for lambs infected with gastro-intestinal parasites

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**Introduction** In recent years there has been an increased interest for breeding lambs resistant to gastrointestinal parasites due to the reduced efficacy of anthelminthics. Furthermore, protein supplementation alleviates the adverse effects of parasitism since more protein is available for the satisfaction of the competing body functions of growth and resistance to parasites. Therefore, differences in dietary protein might result in the expression of genotype x environment interaction. Additionally the estimates of genetic and phenotypic correlations obtained from populations kept under different nutritional conditions might differ with implications for the breeding programmes. The aim here is to explore the effect of dietary protein level on the estimates of genetic and phenotypic correlations of a population of growing lambs infected with gastrointestinal parasites using a simulation model.

**Materials and methods** A simulation model for the interaction between host nutrition, genotype and gastro-intestinal parasitism in individual lambs (Vagenas *et al*., 2007) was extended to population level by introducing genetic and environmental variation in appropriate input traits associated with growth and resistance to parasites. A zero (genetic and phenotypic) correlation between these traits was assumed. Initial variation in the input traits leads to time-dependent genetic and phenotypic variation in and co-variation between production and disease resistance traits. The variation and correlation of these traits will also be affected by the environment i.e. diet. The simulated population (any breed) comprised 10,000 lambs, which were the offspring of 250 sires randomly mated to 5000 dams. For resistance traits, a natural logarithm transformation was used to generate approximately normally distributed traits. Genetic correlations between traits were estimated using a sire model (Falconer and Mackay, 1997). Two diet regimes were simulated: (i) *ad-libitum* good quality grass ('good diet': 12.6 MJ/kg DM ME and 0.19 kg CP/kg DM) and (ii) *ad-libitum* poor quality grass ('poor diet': 7.5 MJ/kg DM ME and 0.097 kg CP/ kg DM). A trickle challenge of 3000 L3 *Teladorsagia* was assumed.

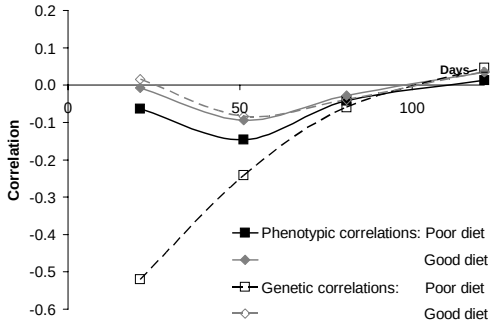


**Figure 1** Genetic and phenotypic correlations between protein retention and faecal egg counts (natural log). The diets have been defined in the text.

**Results** The model predicts that lambs fed on good quality grass grow on average faster than lambs fed on poor quality grass despite an average lower food intake. They are also predicted to have a higher resistance to parasites represented e.g. by worm burden, although they have on average higher faecal egg counts (FEC) due to a lower volume of faeces produced. Figure 1 shows genetic and phenotypic correlations between growth rate, represented by protein retention and the natural log of FEC at 21, 50, 80 and 120 days after first exposure to parasites. The estimated genetic correlations are generally stronger compared to the phenotypic correlations. Except for day 50, genetic and phenotypic correlations are close to zero when lambs are fed good quality grass, indicating that the nutritional demands for growth and resistance functions are fully met. At approximately 50 days after the first challenge, a trade-off between growth and immunity occurs, which is manifested by negative genetic and phenotypic correlations. This trade–off is represented more strongly when lambs are fed poor quality grass, as they cannot simultaneously satisfy the nutritional demand for production and immunity. This results in relatively high negative correlations between protein retention and FEC. This phenomenon is strongest when the lambs are younger and are acquiring immunity against gastrointestinal parasites. As animals become immune at older ages the effect of parasitism on growth diminishes resulting in similar correlations for both nutritional regimes.

**Conclusions** The model results suggest that host nutrition has a significant effect on the genetic and phenotypic relationship between production and resistance traits in lambs that are sub-clinically infected with gastrointestinal parasites. The results match observations from experimental studies (e.g. Bishop and Stear, 1999) and verify existing theoretical frameworks (Coop and Kyriazakis, 1999). In line with Vagenas *et al.* (2007) this model can be further used to explore the interactions between different host genotypes (Scottish Blackface – Suffolk) for production and disease resistance traits and nutrition on performance and immunity. Understanding of these interactions is important for the design of successful breeding strategies.

**Acknowledgements** Financial support of BBSRC and SEERAD is gratefully acknowledged.

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**090**

# How does host nutrition affect the development of a microparasitic infection?

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**Introduction** Models that predict phenotypic responses from the interaction between genotypic descriptors and the environment are desirable both in the context of both animal production systems and evolutionary ecology. Nutrient availability is often related to the ability of a host to control an invading parasitic population and mounting an immune response is often associated with a nutritional cost. This cost is shown by the peripartuient break down of immunity (Houdijk *et al.,* 2003) and reports of negative correlations between production and resistance traits (Rauw *et al.,* 1998). The aim was to develop a model which is able to make predictions relating to the effects of resource allocation as determined by nutrition and genotype, on the course of microparasitic infection in farm animal hosts.

**Materials and methods** The within host coupled differential model of Antia *et al*., (1996) was considered as a suitable basis for model development; it is able to make predictions relating to the size of a pathogen load (*PL*), and the size of an immune response (*I*) through the course of time. This model consists of two differential equations: one describing the change in size of the pathogen load (*EqPL*) and the other the change in size of the immune response (*EqI*). *EqPL* represents the two opposing forces within the host-parasite system, pathogen replication and pathogen clearance by the immune response. *EqI* models the development of the immune response as being dependent upon the size of the current pathogen load up to a maximum. This model was modified to account for the effects of host nutrition and genotype. *EqI* is used to calculate a nutritional requirement for the immune response in a given time step. This requirement, relative to the requirement for growth multiplied by the 'above maintenance' supply of the most limiting nutrient (*N*), gives the realised increase in the immune response. Hosts are assumed to differ in both their requirements for growth and in their potential for disease resistance ($\rho$); $\rho$ is a parameter of *EqI*, the potential maximum *per capita* replication rate of *I*. Simulation was used to investigate the interactive effects of nutritional status and genotype upon various infection characteristics.

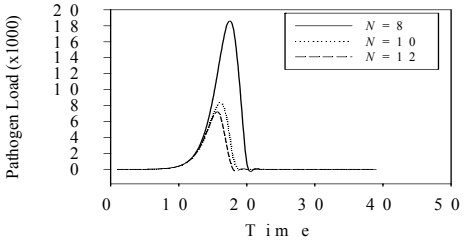


**Figure 1** The predicted effects of nutrient supply (*N)* on the course of an initial pathogen load of 1 (arbitrary units)

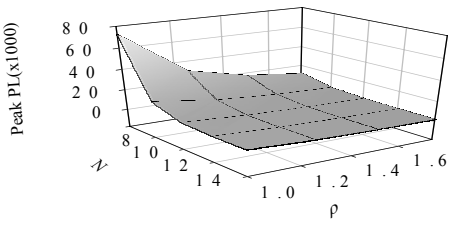


**Figure 2** The predicted effects of nutrient supply (*N)* and $\rho$ on the peak pathogen load reached during an infection (arbitrary units)

**Results** Hosts with a greater nutrient availability were predicted to have lower peak pathogen loads, duration of infection and cumulative pathogen loads, up to the point at which resources become unlimiting (Figure 1). Similarly, hosts with a greater $\rho$, had reduced infection characteristics. The $\rho$ (genotype) x *N* (environment) interaction is apparent in Figure 2. Under conditions of severely limiting resources hosts with a greater degree of genetic resistance gain a significant advantage in terms of limiting the consequences of infection. However, such a difference is reduced, though still present, when resources become unlimiting. Host growth potential is predicted to be negatively related to the severity of infection, but only under states of poor nutrient availability.

**Conclusion** The developed model is a step towards a further understanding of the interactive effects of nutrient availability and host genetic potential upon observed disease resistance and growth. In general the predictions of the model are in agreement with empirical findings. The model may be used to explore the existence of any phenotypic, genetic and environmental correlations between aspects of disease resistance and growth. There have been conflicting reports upon the sign of such correlations (Knap and Bishop, 2000). The resource allocation model proposed would suggest that the sign and strength of the correlations would be dependent upon resource availability.

**Acknowledgements** WB was in receipt of a studentship from SAC Trust Funds. SAC receives support from the Scottish Executive, Environment and Rural Affairs Department.

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**091**

# Effects of secondary nematode infection on anorexia and leptin levels in growing lambs of two different breeds

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**Introduction** A primary infection of a parasite naïve animal has detrimental effects on intake (anorexia), which in turn results in impaired animal productivity. Recent studies have indicated that anorexia in nematode infected lambs is a direct consequence of the acquisition of immunity (Greer *et al.,* 2005). In many models of disease, immune system activation results in elevated leptin levels and these have been associated with anorexia. However, whether the expression of acquired immunity following a secondary nematode infection results in elevated leptin levels and/or anorexia in growing lambs it is not known. In addition, it is not known whether the expression of acquired immunity differs between breeds that differ in production potential. The aim of the present study was to test the hypotheses a) that a secondary nematode infection results in increased leptin levels and anorexia in growing lambs and b) that lambs of a high production potential breed exhibit a higher degree of anorexia than lambs of a low production potential.

**Materials and methods** Forty-eight immune lambs (~6 months of age), half Suffolk × Greyface (S) crosses (representing the higher production potential breed) and half Scottish Blackface (B) that had been receiving infection with 21,000 infective larvae of *Teladorsagia circumcincta* (TC) for a period of 12 weeks, were used in the trial. At the end of primary infection, animals were drenched to eliminate parasites. Two weeks later, half of the lambs of each breed were either dosed with 7,000 infective third-stage TC larvae ($L_3$) three times per week (Monday, Wednesday and Friday) and fed *ad libitum* grass pellets (GP*)*, or not re-infected and fed GP either *ad libitum* (n=8) or restrictively at 90% of *ad libitum* (n=4). The trial lasted for 12 weeks and food intake (FI) and faecal egg counts (FEC) were recorded weekly. Blood samples were taken every fortnight and analyzed for plasma leptin concentrations using a homologous ovine-specific RIA. REML was used to analyze leptin (after log-transformation) and FI. Log transformed FEC were analyzed using ANOVA with repeated measurements.

**Results** There was a significant difference in mean daily FI between the two breeds but the relative FI (RFI, $g \cdot kg^{-1} \cdot d^{-1}$) was not different between the breeds. Re-infection did not cause a significant reduction in FI in the Blackface lambs ($P$= 0.58). However, the effect of re- infection was significant ($P$= 0.05) in the Suffolk lambs which became more clear from the ninth week of re-infection and onwards (Fig. 1a). Group mean FECs remained very low (below 15 e.p.g.) over the re-infection period and were not affected by breed. Leptin levels were affected significantly by infection ($P$= 0.01), however, the difference in leptin levels was significant only between infected and non-infected Suffolk lambs ($P$= 0.03). Leptin levels also differed significantly between the two breeds ($P$= 0.03) (Fig. 1b and 1c).

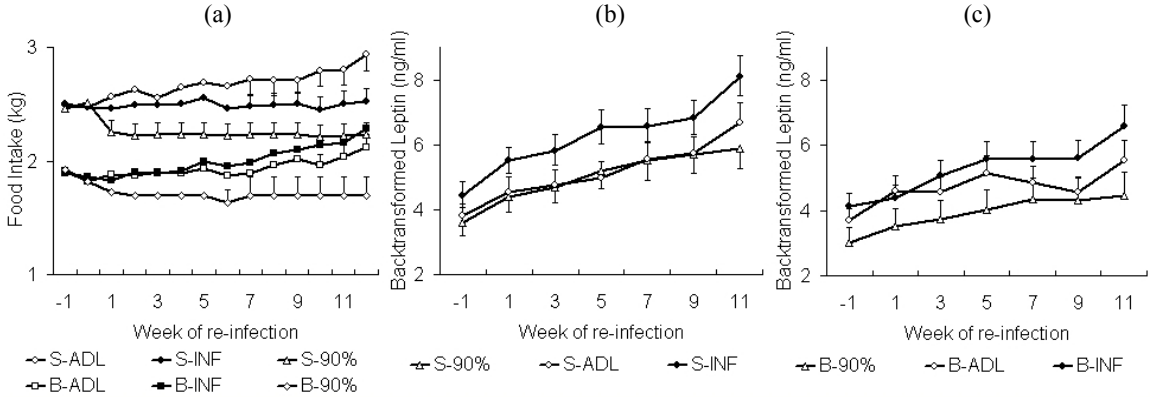


**Fig. 1** Daily average FI (1a) and back-transformed means of leptin in Suffolk (b) and Blackface lambs (c).

**Conclusion** FECs remained very low, which suggests that a period of two weeks between a primary and a secondary infection, during which animals are not infected, does not result in significant loss of acquired immunity in growing lambs. Re-infection caused a significant reduction in FI in Suffolk lambs, which might suggest that the expression of the acquired immunity results in anorexia. In addition, the higher leptin levels in the infected lambs in comparison to the non-infected may suggest that immune response is associated with leptin. If that is the case, then leptin might have a role in the parasite induced anorexia. Further studies that will involve direct measurement of immunity (e.g. IgA) and leptin will elucidate whether leptin concentration correlates positively with the development of immunity in nematode infected lambs.

**Acknowledgements** This research was financially supported by SEERAD. Mr Konstantinos Zaralis is grateful to the Hellenic State Scholarships Foundation for the provision of a postgraduate scholarship.

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# Interactive effects of selection for growth and protein supply on the consequences of gastrointestinal parasitism on growth performance in mice.

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**Introduction** The consequence of sub-clinical gastrointestinal parasitism in farm animals, i.e. reduced food intake and growth, is often more pronounced in breeds with a high production potential compared with breeds with a low one (Houdijk *et al.* 2001). It cannot be excluded that such differences in disease resistance may arise from between-breed differences in genetic resistance to parasites rather than production potential *per se*, as within-breed selected farm animals with sufficiently different production potentials are not readily available. However, appropriately selected mouse lines are available (Bünger *et al.*, 2001) to explore the hypothesis that selection for growth may reduce the animal's capacity to cope with pathogens. We have recently shown in mice divergently selected for growth potential that in some lines, the absolute penalty of sub-clinical parasitism on high growth mice was higher than in their low growth counterparts (Houdijk and Bünger, 2006). Here, we test the hypothesis that this penalty on growth in one of these lines can be reduced through increased protein supply.

**Materials and methods** The experiment consisted of a 2x2x2 factorial design (n=8), with two levels of growth potential, infection and dietary protein content. Weaned male mice were derived from the Roslin lines that had been divergently selected for high (ROH) or low (ROL) growth potential over at least twenty generations (Bünger *et al.*, 2001). At day 40 of age, mice were either dosed with 250 infective larvae of the intestinal nematode parasite *Heligmosomoides polygyrus* or sham-infected with water (day 0). All mice were individually housed and had *ad libitum* access to iso-energetic foods formulated to contain either 50 (LP) or 250 (HP) g crude protein per kg dry matter. Food refusals and body weight were assessed twice weekly until day 28 to estimate body weight gain and food intake. Faeces were collected on day 21 and 25 to assess the concentration of nematode eggs. The latter data were log-transformed before statistical analysis, and data were analysed through a 2x2x2 ANOVA.

**Results** Infection interacted with growth potential ($P<0.001$) for food intake (Figure 1). Infection significantly reduced intake for the ROH mice but not for the ROL mice, and tended to be more pronounced in LP mice ($P<0.001$) than in HP mice ($P<0.05$). The three-way interaction between growth potential, infection and protein nutrition was significant for body weight gain ($P<0.01$; Figure 1). Infection reduced body weight gain in ROH mice ($P<0.001$), but not in ROL mice, whilst within ROH mice, this penalty was smaller for HP mice than for LP mice ($P<0.001$). Protein supply did not affect the concentration of nematode eggs in the faeces but the latter was higher for ROL mice than for ROH mice ($P<0.01$).

**Conclusion** The results support the view that selecting for growth performance in the Roslin line may increase the absolute penalty of parasitism on performance, and that increased protein supply can ameliorate this penalty to some extent. Such information would be important when using a narrow breeding goal, for example, selecting for increased growth performance only. However, these interactive effects of growth potential and protein nutrition were not consistent with observed effects on the concentration of nematode eggs in the faeces, although this may have been related to differences in faeces volume between ROL and ROH mice. The absence of effect of dietary protein contents on growth in non-infected mice indicate that protein requirements for maintenance and growth can be satisfied from low protein foods through increasing food intake. The latter did not occur in the infected mice, which indirectly suggests that gastrointestinal nematode parasitism significantly increases host protein requirements.

**Acknowledgements** This work was supported by SEERAD.

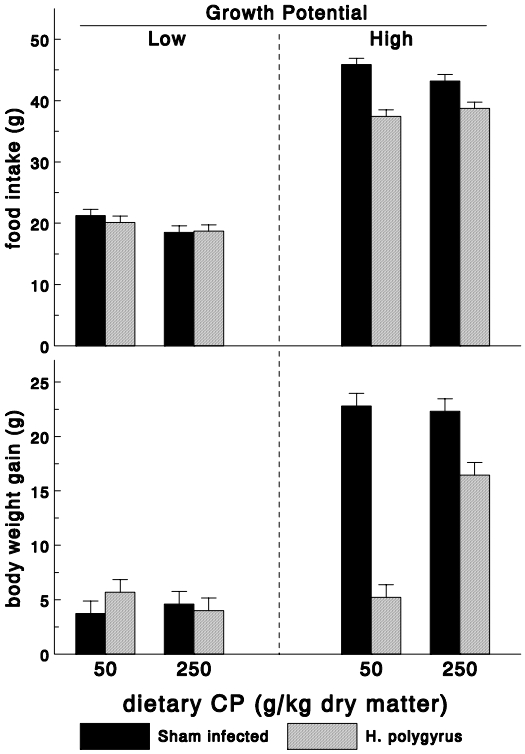


**Figure 1** Mean food intake and body weight gain over 28 days in weaned male mice of low and high growth potential, offered low or high protein foods and infected with *H. polygyrus* or sham infected with water.

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# Temporal effects of protein supply on expression of immunity to Nippostrongylus brasiliensis during lactation in rats

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**Introduction** Expression of acquired immunity to gastrointestinal parasites usually breaks down during the periparturient period, which is characterised by an increased worm burden and nematode egg excretion. A possible nutritional basis of this phenomenon has been addressed in a rat model, as lactating rats exhibit a breakdown of immunity to the gastrointestinal nematode *Nippostrongylus brasiliensis* (Houdijk *et al*., 2005). Indeed, increasing protein supply and reducing protein demand at times of protein scarcity improved resistance to *N. brasiliensis* (Houdijk *et al*., 2005; Normanton *et al*., 2006) but did not affect associated local immune responses. The latter may have been due to the single sampling point used. Therefore, the objective of the current experiment was to assess temporal effects of increased protein supply on resistance and immune responses to *N. brasiliensis* in lactating rats.

**Materials & methods** 48 second parity female Sprague-Dawley rats were given a single dose of 1600 third-stage infective *N. brasiliensis* larvae. Post primary infection, females were mated and during pregnancy, all rats were fed similarly. Parturition was considered as day 0, and from then onwards rats were restrictedly offered (at 7.5% of their parturition body weight) either a low protein diet (LP, 100g CP/kg DM) or a high protein diet (HP, 300g CP/kg DM). Litter size was standardised to 9 pups by day 2 of lactation. The rats were re-infected with a single dose of 1600 *N. brasiliensis* larvae on day 2. Groups of rats were slaughtered on day 5, 10 or 15 post secondary infection for the assessment of the concentration of nematode egg counts in the colon (CEC) and the concentration of four types of immunoglobulin (IgA, IgE, $IgG_1$ and $IgG_{2a}$) and rat mast cell proteases (RMCP-II, an indicator of inflammatory cell activity) in small intestinal mucosal scrapings. Immune responses and CEC were log transformed, prior to ANOVA, and are reported as backtransformed means with backtransformed lower and upper standard errors.

**Results** Feeding treatment did not affect feed intake. HP and LP rats had lost 38g and 65g, respectively (SED: 3.90; P<0.001), and their litters had gained 169g and 103g, respectively (SED: 8.58; P<0.001) by day 15. Diet and time did not significantly interact for CEC (Figure 1; P=0.48) and for RMCP-II (Figure 2; P=0.38). HP rats tended to have lower CEC (P=0.002) and had significantly lower concentrations of RMCP-II (P<0.001) than LP rats, with effects on RMCP-II being most pronounced on day 10 and 15 (P<0.05). There was a trend for diet and time to interact for IgA (P=0.11), IgE (0.10) and $IgG_{2a}$ (0.12) levels, with HP rats tending to have higher levels on day 5 compared to LP rats (P=0.07). There was no interaction between diet and time for $IgG_1$ levels (P=0.29).

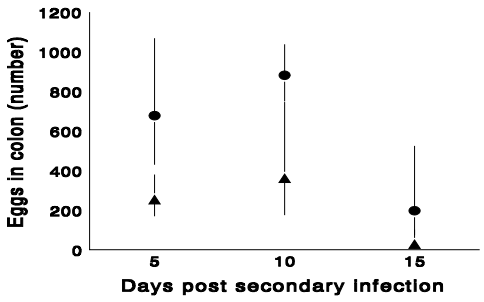


**Figure 1** Colon egg count on day 5, 10 & 15 post secondary infection for rats receiving high (▲) or low protein (•) diets.

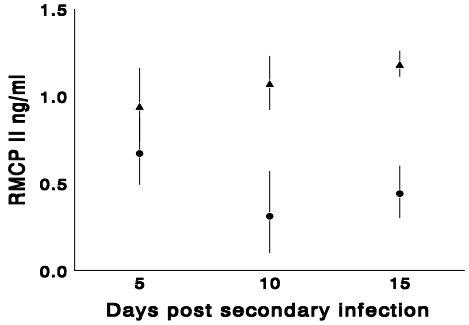


**Figure 2** The level of RMCP-II present the mucosa from rats receiving high (•) or low protein (▲)diets.

**Conclusion** The results support the view that increasing protein supply at times of protein scarcity improves periparturient resistance to *N. brasiliensis*, as illustrated by the lower number of nematode eggs in the colon. Although effects of increased protein supply on some immune responses were not statistically significant, these novel findings suggest that nutritional sensitivity of selected immune responses associated with periparturient resistance to *N. brasiliensis* may differ over time relative to challenge infection. The reduced concentration of RMCP-II in the HP rats may indicate that protein supplementation leads to reduced degree of mucosal inflammation as a consequence of reduced parasitism. However, further research is necessary to fully understand the underlying immunological basis of relaxation in immunity during the periparturient period and its sensitivity to nutrient scarcity.

**Acknowledgements** This work was supported by SEERAD.

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Normanton, H., Houdijk, J.G.M., Knox D.P. and Kyriazakis, I. (2006). The effect of changes in nutrient demand on gastrointestinal parasitism in lactating rats. *British Journal of Nutrition* (in press).

# Effects of protein supplementation on anorexia and expression of immunity in parasitized periparturient ewes of two different breeds

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**Introduction** The breakdown of acquired immunity during the periparturient period in the ewe can be reduced by metabolizable protein (MP) supplementation (Houdijk *et al* 2001). Recent evidence suggests that the development of immune response results in reduced food intake (anorexia) in many disease models (Materase *et al,* 2005). However, it is not known whether an immune response following the periparturient relaxation of immunity is associated with a reduction in food intake and whether this is affected by protein supplementation in parasitized ewes. In addition, differences in nutrient partitioning between sheep breeds that differ in production potential may affect the ability of the hosts to express immunity and this may be reflected by differences in their magnitude and/or duration of anorexia. The aim of the present study was to test the hypotheses that: a) nematode infection during the periparturient period results in anorexia and protein supplementation can effect the degree of anorexia in ewes, and b) ewes of a high production potential breed show higher breakdown of acquired immunity than ewes of a low production potential breed, and exhibit higher degree of anorexia.

**Materials and methods** A 2×2×2 factorial design was used with two breeds of twin-bearing ewes (Scottish Blackface, B (n=32) and Greyface cross, G (n=32)), two levels of protein supplementation and two levels of infection. Ewes in both low (B) and high (G) production potential breeds were either trickle infected (+) with 30,000 infective *Teladorsagia circumcincta* larvae per week or not infected (non INF) (-), from six weeks before until four weeks after parturition. All ewes were fed with a pelleted diet which provided 75% of their metabolizable protein (MP) requirements (LP). An additional protein supplementation (soypass) that enhanced the MP content up to 125% of the ewes' requirements was offered to half of the ewes (HP) in each infection treatment. The experimental diets were offered from four weeks before until four weeks after parturition. Food intake (FI) and faecal egg count (FEC) were recorded twice weekly. Blood samples were taken weekly for pepsinogen analysis. FI data were analyzed using REML. FEC and pepsinogen analysed by ANOVA after log-transformation.

**Results** Nematode infection caused a significant reduction ($P<.0001$) in FI in periparturient ewes (Fig 1a). Protein supplementation enhanced FI ($P=0.001$) but did not affect significantly the extent of anorexia ($P=0.30$). No significant difference was observed in FI between the two breeds ($P=0.6$). On average, mean daily intakes were 2.31, 1.84, 1.9 and 1.4 kg·day$^{-1}$ (SE 0.11) in the HP-, HP+, LP- and LP+ treatments, respectively. FEC analysis showed that there was a significant breed difference attributable to the higher FEC in the G breed. Protein supplementation resulted in significant lower FEC in both breeds (Fig 1b). Pepsinogen levels affected significantly by infection ($P<.0001$) in both breeds but not by protein supplementation (Fig 1c).

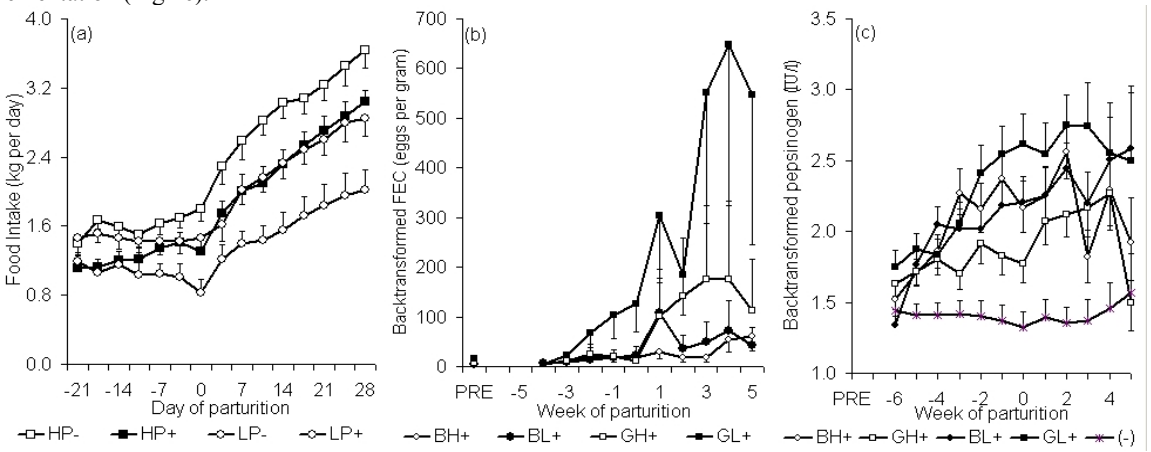


**Figure 1** Daily average FI (a), backtransformed means of FEC (b) and plasma pepsinogen concentration (c). Standard errors (SE) are also showed.

**Conclusion** Infection of immune ewes with *T .circumcincta* during the periparturient period resulted in a reduction in food intake. The study confirmed the view that protein supplementation can assist periparturient ewes in controling parasite infection, as judged from FEC, but had no effect on the degree of anorexia. FECs, as an indirect indicator of immune response, were lower in the Scottish Blackface than in the Greyface ewes, suggesting that these breeds have significant differences in their ability to control nematode infection. However, the significant effect of breed on FEC was not associated with a significant difference in the degree of anorexia. Further research that involves measurement of blood parameters such as leptin, could provide novel data on the mechanism underlying the parasite induced anorexia in sheep.

**Acknowledgements** This research was financially supported by SEERAD. Konstantinos Zaralis is grateful to the Hellenic State Scholarships Foundation for the provision of a postgraduate scholarship.

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# The effectiveness of copper oxide wire particles as an anthelmintic in purebred Suffolk lambs exposed to a natural nematode challenge post weaning.

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**Introduction** Resistance to nematode parasites of sheep to broad spectrum anthelmintics is one of the main drivers in research on alternative solutions for parasite control. Previous work has shown clear differences between the Suffolk and Texel breeds in susceptibility to gastrointestinal nematode infection (Hanrahan & Crowley, 1999; Good, Hanrahan *et al*., 2006). The Suffolk being more susceptible to such parasitic infections has implications in the Irish industry where it plays a dominant role as both as a terminal sire and in the genetic makeup of the ewe population. Previous work has shown some beneficial anthelmintic effects following the administration of copper oxide wire particles (Bang, Familton *et al*., 2000; Knox, 2002). The aim of this study was to evaluate the potential of copper oxide wire particles (COWP) as an anthelmintic for lambs that were exposed to a natural nematode challenge postweaning.

**Materials and methods** Purebred Suffolk lambs (n=24) were randomly assigned to one of 3 treatment groups postweaning; A: administered levamisole (5 ml/10 kg; Nilzan Drench Plus, Schering Plough Animal Health) plus COWP (2.0 g/lamb; Copasure, Animax Ltd.), B: COWP and C: control (no COWP or levamisole administered) and cograzed. Strongyle faecal egg counts were determined for each lamb on a weekly basis. Faecal egg counts were distinguished as *Nematodirus* ($FEC_{NEM}$), *Strongyloides papillosus* ($FEC_{Spap}$) and 'other trichostrongyles' ($FEC_{OT}$). Coprocultures for each treatment group were also completed to determine information on parasite species compositions of the FEC. Animals were weighed pre and post trial. FEC (weeks 2, 3 & 4) was analysed using Proc Mixed of SAS (SAS, 1989) with animal within treatment as a random effect and initial FEC (taken pre-treatment) used as a covariate. The model had fixed effects for treatment, week and treatment-by-week interaction. Prior to analysis, the FEC data were transformed to logarithms (ln (x +1)) to stabilise the variance.

**Results** On the basis of rising FECs and growing clinical evidence of infection it was decided to shorten the trial by 2 weeks and all animals were treated with levamisole at week 4. Least squares mean (weeks 2, 3 & 4) for $FEC_{NEM}$, $FEC_{Spap}$ and $FEC_{OT}$ according to treatment group are shown in Table 1. There was a significant treatment effect for $FEC_{Spap}$ ($P<0.05$) and $FEC_{OT}$ ($P<0.01$). There was a significant week and treatment-by-week interaction for $FEC_{OT}$ ($P<0.005$). The treatment by week means for $FEC_{NEM}$, $FEC_{Spap}$ and $FEC_{OT}$ are shown in Figure 1. Larvae recovered from the coprocultures were predominantly species that reside in the small intestine. There was no treatment effect on final weight. The mean weight (± s.e.) for animals in treatment groups A, B and C were 51.2 ± 0.78, 50.5 ± 0.82, 48.6 ± 0.92 kg respectively.

**Table 1** Least squares means and standard errors (log scale) (weeks 2,3 & 4 ) of faecal egg counts by treatment

| | Treatments | | |
|---|---|---|---|
| | Levamisole + COWP | COWP | CONTROL |
| $FEC_{NEM}$ | 2.4 [a] (0.69) | 3.4 [a] (0.68) | 3.1 [a] (0.76) |
| $FEC_{Spap}$ | 3.7 [a] (0.26) | 4.9 [b] (0.26) | 4.3 [ab] (0.28) |
| $FEC_{OT}$ | 6.4 [a] (0.20) | 7.4 [b] (0.20) | 7.3 [b] (0.22) |

[ab] Means with different superscript letters, within rows are significantly different ($P< 0.05$)

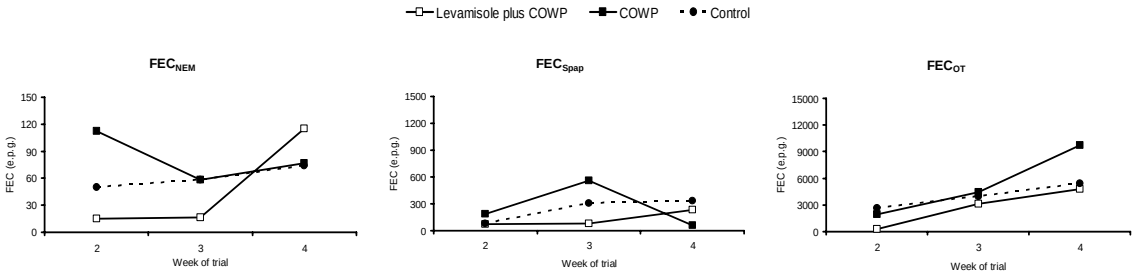


**Figure 1** Least squares means (back transformed) for FEC at each time point

**Conclusions** The results of the present study indicate that administration of copper oxide wire particles in lambs post-weaning had no effect on the faecal egg count. However, the main species identified from faecal cultures were those located in the small intestine which, by their location, would not be susceptible to the effects of COWP.

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# Effects of an algal biomass supplement on the periparturient rise in nematode egg output from ewes and the subsequent effects on their offspring

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**Introduction** The periparturient rise in nematode eggs at lambing is a crucial part of the epidemiology of nematode infections as it allows a large number of infective larvae to be present in the pastures when the lamb is young and most immunologically naïve. With a rapidly rising level of anthelmintic resistance and increasing public concern over drug residues remaining in animal products, it is necessary to achieve more long-term, sustainable nematode control programmes. Some plant products could provide alternatives or adjuncts to conventional control with anthelmintics and ease the selection pressure for anthelmintic resistance. The polyunsaturated fatty acid, docosahexaenoic acid (DHA), which can be produced in high concentrations by some marine algae, has been shown to modify immune response in many species and decrease the production of proinflammatory cytokines (Calder, 2001). This action could reduce inflammation in the gut in response to parasite infestation and reduce the extent of associated hypophagia. The objective of this study was to investigate the long term effects of providing an algal DHA dietary supplement in late pregnancy, on the periparturient rise and the resulting effects on the infection levels and production consequences for their lambs.

**Materials and Methods** 40 twin bearing mule ewes were allocated according to faecal egg counts (FEC) and age to a 2x2 factorial design comparing presence or absence of (1) an herbal anthelmintic product and (2) an algal biomass (AB) supplement. Treatment ewes received 32g of algal biomass providing 6g of DHA per day for the last 6 weeks of pregnancy. Faecal and blood samples (for complete blood count) were taken on day zero and then weekly for a total of 15 weeks. Lambs were weighed at birth, 24 post partum and week 6 and blood samples were taken at 24 hours post partum for complete blood count. Stock were grazed in the same split field with groups plus and minus AB grazed together within anthelmintic treatment. The effect of the herbal product will not be further discussed (due to confidentiality agreement) but was taken into account in evaluating the effect of algal biomass supplementation. Parametric data and log transformed FEC were analysed using two-way analysis of variance, with herbal treatment, AB and their interaction as factors. Non parametric data were analysed across all treatments using the Kruskal Wallis Test, with subsequent pairwise comparisons by Mann Witney Tests.

**Results** There were no significant effects of algal biomass supplementation on ewe haematology, condition score (CS) change prior to lambing or faecal egg count at the time of turnout. There were also no significant effects on lamb weight at birth, haematology or liveweight gain at 24 hours post partum, or liveweight gain from birth to 6 weeks post partum (Table 1), or any interactions between AB and herbal product in these measures.

**Table 1** Effects of Biomass supplementation on ewe and lamb measures

| | - AB | +AB | SEM | P value |
|---|---|---|---|---|
| Ewe FEC at Turnout (log eggs/g) | 2.53 | 2.51 | 0.106 | NS |
| Ewe CS change (1-5 scale) | -0.3 | -0.5 | 0.10 | NS |
| Lamb Birth Weight (Kg) | 5.28 | 4.94 | 0.152 | NS |
| Lamb lwt gain to 24 hours (Kg) | 0.18 | 0.14 | 0.064 | NS |
| Lamb daily lwt gain to 6 weeks (Kg) | 0.28 | 0.27 | 0.012 | NS |

At 6 weeks post partum the experiment had to be terminated because of a severe coccidial infection in the lambs. Faecal dag scores (0= clean to 3= very dirty) were taken blind of treatment from each lamb at this time to estimate the severity of infection. There was a significant treatment difference in the dag scores of the lambs (Kruskal Wallis Test H= 11.59, 3 df, p = 0.009). Mann Whitney pairwise comparisons showed the AB supplementation group without herbal product (median 0, IQ range 0-1) to be significantly different to all other treatment groups (medians 1, 1.5 and 2) which did not differ significantly from each other.

**Conclusion** Algal biomass supplementation showed no beneficial effects prior to termination of the experiment but appeared to reduce the severity of coccidial infection when given alone, but not in combination with the herbal anthelmintic. Kumaratilake et al. (1992) showed marked parasiticidal properties of polyunsaturated fatty acids, especially DHA, resulting in intraerythrocytic degeneration and death of the malarial parasite *P. falciparum*. They noted that parasite killing was significantly increased when using previously oxidised forms of fatty acids and was decreased in the presence of antioxidants. It is hypothesised that the higher levels of DHA in the milk of the ewes previously fed the algal biomass may have had a similar influence on coccidial viability, an effect inhibited by the antioxidant properties of the herbal supplement. This observation merits further study.

**Acknowledgments** This experiment was funded by The Red Meat Industry Forum. We thank ABN (Europe) and Ellen Collinson Herbs for provision of test materials.

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# Categorisation and analysis of risks affecting the prevalence of lameness in dairy cattle

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**Introduction** Lameness as a herd problem in dairy cattle typically presents as lesions of the claw horn (CHL); e.g. sole ulcer and white line disease, or as digital dermatitis (DD), an infection of the adjacent skin. The aetiology of these conditions is complex and over 100 potential hazards have been identified involving features of the environment, nutrition, phenotype and physiological state. Strategic plans for control of lameness need to be farm-specific and address the risks attached to these potential hazards on an individual basis. For purposes of analysis however, it is possible to allocate the multiplicity of hazards into functional categories: e.g. external forces acting on the foot, infection and hygiene, foot structure, systemic disorders. This paper examines associations between the severity of proximate risks ascribed to these hazard categories and the prevalence of lameness in first-calving heifers.

**Materials and methods** The study was carried out on 60 farms identified as having a lameness problem.. Each farm was visited twice over a three-year period. Potential hazards for CHL and DD were recorded and categorised. The Proximate Risk for each hazard category was quantified as a score (0-4). 1197 heifers in the first 120 days of their first lactation were locomotion scored (LS) on a 6-point scale and their hind feet inspected for lesions. Animals with locomotion score 0 were classified as sound, all those with scores 1-5 were termed unsound and those with scores 4-5 described as severely lame. Associations between proximate risks and the prevalence of unsoundness and severe lameness were analysed for significance and ranked in order by Spearman Rank correlations.

**Results** The overall prevalence of lameness, as assessed from locomotion score, was 44% sound, and 56% unsound, of which 15% were severely lame. CHL were observed on all farms and DD on all but three farms. Risks from proximate hazards are ranked, with correlations in the following table. The three highest ranking risks for unsoundness were factors that cause claw trauma, suboptimal claw condition at calving and wet slurry underfoot. There was no association between evidence of rumen disorders and unsoundness. Breach of biosecurity was not a significant risk for DD in this study because DD was already endemic. The ranking of risks for severe lameness presented a very different picture, the most important being deficiencies of foot care; e.g. suboptimal claw condition at calving, and deficiencies in routine preventive medicine.

**Table 1** Ranking of proximate risks for unsoundness and severe lameness in dairy heifers.

| | Proximate risk | Unsoundness | | Severe lameness | |
|---|---|---|---|---|---|
| | | Rank | Coefficient | Rank | Coefficient |
| Claw horn lesions | Factors that cause claw trauma | 1 | 0.42 | 5 | 0.29 |
| | Suboptimal claw condition at calving | 2 | 0.41 | 1 | 0.46 |
| | Prolonged standing on concrete | 5 | 0.35 | 6 | 0.28 |
| | Delayed detection and treatment | 6 | 0.32 | 2 | 0.41 |
| | Rumen disorders | 13 | n.s. | 11 | 0.20 |
| Digital dermatitis | Wet slurry underfoot | 3 | 0.41 | 12 | n.s. |
| | Prolonged standing in slurry | 4 | 0.36 | 17 | n.s. |
| | Ineffective prevention and treatment | 14 | n.s. | 3 | 0.34 |
| | Breaches of biosecurity | 15 | n.s. | 8 | 0.28 |

**Conclusions** The most important proximate risks for unsoundness in dairy heifers were those relating to the physical environment; trauma, prolonged standing on concrete and in wet slurry. There was no evidence for systematic effects associated with rumen disorders. Progression from unsoundness to severe lameness associated with e.g. sole ulcer or active DD was primarily associated with failures in preventive foot care.

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# Effect of lameness on the daily milk yield of Holstein-Friesian dairy cows

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**Introduction** As a major health problem facing dairy farmers worldwide with substantial economic, production and welfare consequences, lameness has attracted much attention over the last two to three decades (Boelling, 1999). Lameness as a linear type trait is measured by locomotion scoring as there is a high genetic correlation between lameness and locomotion score. Studies have included locomotion (as a predictor of lameness) and have associated lameness with no effect, an increase and sometimes a decrease in milk production of cows. Several reasons have been given for these differences and the need still arises for estimates of the effect of lameness on milk yield in herds with current dairy management. The objective of this study was to quantify the effect of lameness on the daily milk yield (DMY) of dairy cows.

**Materials and methods** Daily milk yields and weekly locomotion scores on 248 cows from the Langhill herd at Crichton Royal Farm in Dumfriesshire, Scotland were used for the study. The locomotion scoring technique used is as described by staff at Crichton Royal Farm (1-5 scale), where 1 denotes "perfect" and 5 reflects "difficulty in turning". The herd is representative of a high producing dairy herd. Data were obtained from 5 lactations and animals were within 1 and 350 days in milk (DIM). Cows were randomly allocated to two management regimes – XE (cows fed a high concentrate and low forage diet) and XM (low concentrate and high forage diet). Cows were grouped into heifers (all 1$^{st}$ lactation cows) and cows (2$^{nd}$, 3$^{rd}$, 4$^{th}$ and 5$^{th}$ lactation cows) as there were few 3$^{rd}$ (106), 4$^{th}$ (54) and 5$^{th}$ (20) lactation animals and analysed separately. Milk yield was analysed including lactation number, feed group and locomotion score as explanatory variables. A further investigation examined differences in the shape of lactation curves of cows judged lame (3, 4 or 5) and those judged never lame (scored 1 or 2). Lame cows were further categorized into those that became lame on or before 60 DIM and those lame after 60 DIM. Analysis was done using residual maximum likelihood in the software package R (Venables *et al*., 2005).

**Results** Locomotion score did not influence the milk yield of heifers significantly ($P > 0.05$). As a result differences in lactation curves for lame versus non-lame heifers were not studied. The milk yield of the cows was significantly ($P < 0.05$) affected by locomotion score. Result (Table 1) showed that a locomotion score of 4 was associated with a 1.11kg loss in DMY compare to cows that scored 1, while a score of 5 was associated with a reduction of 5.41kg. Figure 1 indicates that the highest yielding cows became lame early in lactation (before 60 DIM). Cows of average production were lame after 60 DIM while lower yielding cows were never lame throughout lactation. Although all coefficients were significantly different from zero, an F-test for these curves showed a highly significant difference ($P < 0.001$) in the linear and quadratic coefficients but not between cubic coefficients. These differences reflect a significant variation in the shape of the lactation curves rather than merely the height of the curves.

**Table 1** Least square mean estimates of explanatory variables for milk yield (kg) of heifers and cows

| | | Locomotion score | | | | Days in milk | | | |
|---|---|---|---|---|---|---|---|---|---|
| Milk yield | Mean | 2 vs 1 | 3 vs 1 | 4 vs 1 | 5 vs 1 | DIM | $(DIM)^2$ | $(DIM)^3$ | XM vs XE |
| Heifer | 26.0 | $-0.023^{ns}$ | $-0.107^{ns}$ | $0.072^{ns}$ | - | $-9.41^{***}$ | $-3.083^{***}$ | $4.82^{***}$ | $-6.97^{***}$ |
| Cow | 30.2 | $0.33^{*}$ | $0.134^{ns}$ | $-1.11^{***}$ | $-5.41^{*}$ | $-14.28^{***}$ | $-2.37^{***}$ | $5.77^{***}$ | $-3.73^{***}$ |

DIM as included in the model = (actual days in milk – 175)/175 and so values lie between ±1. DIM, $(DIM)^2$ and $(DIM)^3$ are linear, quadratic and cubic terms, respectively.

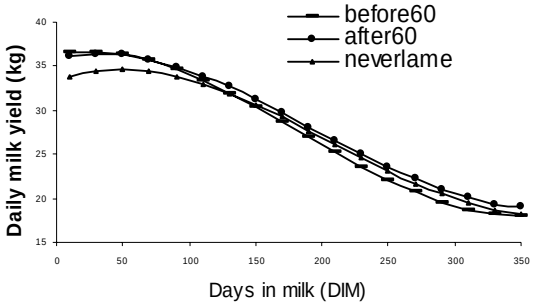


**Figure 1** Milk production curves for lame vs non-lame cows

**Conclusion** Lameness adversely affects the milk production of dairy cows, and high yielding cows are more prone to lameness. This stresses the need for inclusion of health and welfare-related traits as well as production traits in selection indices for optimum herd improvement.

**Acknowledgement** We gratefully acknowledge SEERAD for funding the farm work of this study.

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# A comparison of sole lesion development for Norwegian Dairy Cattle and Holstein Friesian dairy cattle on three different systems in lactation 1 and 2

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**Introduction** Sole lesions and lameness are significant problems for dairy cow production and welfare. Producers are increasingly incorporating non-Holstein genetics into the make-up of dairy herds for a number of reasons, particularly to promote improved fertility and health. However, much of the evidence that alternative breeds improve hoof health characteristics is anecdotal. The aim of the present study was to assess the effects of Holstein Friesian (HF) and Norwegian dairy cattle (N) genotypes on lameness parameters in dairy cattle within different production systems.

**Materials and methods** Following calving, HF (n = 39) and N (n = 45) heifers were allocated to one of three dietary treatments (high level of concentrate ("High"), low level of concentrate ("Low"), and grass-based ("Grass"), referred to as "Diet" in the model). Treatments were balanced for breed. In Lactation 1 and 2 animals on the "Low" treatment were offered a diet of grass silage and concentrate at a ratio of 70:30 and 65:35, respectively for the first 100d of lactation. After 100d of lactation the grass silage to concentrate ratio changed to 80:20 and 75:25, for Lactations 1 and 2 respectively. Animals offered the "High" treatment received a diet of grass silage and concentrate at a ratio of 40:60 and 35:65 for the first 100d of Lactation 1 and 2 respectively. Similar to the "Low" cows, the proportion of concentrates was reduced 100d post-calving to 50:50 and 45:55, in Lactations 1 and 2 respectively. "High" and "Low" animals were continuously housed indoors on a rotational system so that they spent similar amounts of time on slatted and solid concrete floors and were exposed to similar conditions produced by automatic scrapers. Animals on the "Grass" treatment grazed from Spring to Autumn in both years of the study, so that most animals on this treatment grazed from around peak to late lactation. Pathways used by "Grass" cows when moving to and from the parlour were mainly stone/dust lanes with short segments on grass and concrete. While housed, "Grass" cows were offered a diet based on grass silage with a low level of concentrate supplementation. In Lactation 1 "Grass" cows were offered a diet with a grass silage to concentrate ratio of 55:45 from calving to turnout. In Lactation 2 "Grass" cows were offered a total mixed ration with 9 kg of concentrates per day and fresh grass silage. Both hind hooves of each animal were scored for sole lesions 4 times during both the first and second lactations, at 4 observation periods during lactation as follows: (1) -8 to 70d post-calving, (2) 71 to 150d post-calving, (3) 151 to 225d post-calving, and (4) 226 to 364d post-calving. Sole lesions were scored for severity and extent of the hoof affected, using the methodology described by Livesey *et al.* (1998) and the hoof map described by Greenough and Vermunt (1991). Lesion scores over the 6 zones of the sole were added to obtain cumulative lesion scores for the whole claw (zones 1 to 6, "total lesion score") and for the sole (zones 4 to 6) and white line (zones 1 to 3) separately. Scores for both hind claws were added so that each animal had one score. Data were analysed using each observation as a repeated measure in a REML variance components analysis with Lactation, Period (during lactation), Diet, Breed and interaction terms as fixed effects.

**Results** All cumulative lesion scores were higher in Lactation 1 than in Lactation 2 ($P < 0.001$ for total, sole, and white line lesion scores). Total cumulative lesion scores were highest in Period 2, which corresponds with peak lactation. Breed and Diet effects are shown in Table 1. HF cows had higher total lesion scores and higher white line lesion scores than N. Cows on the "Grass" treatment had higher total lesion and sole lesion scores compared to the "Low" treatment. There were no significant interactions between breed and diet.

**Table 1** Breed and diet effects on hoof lesion scores

| | Breed | | | | Diet | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | HF | N | s.e.d | *P* | Grass | High | Low | s.e.d | *P* |
| Total Lesion Score | 11.46 | 9.38 | 1.30 | 0.047 | 12.4[b] | 10.16[a,b] | 8.71[a] | 1.58 | 0.023 |
| Sole Lesion Score | 6.1 | 4.99 | 0.81 | n.s. | 6.95[b] | 5.23[a,b] | 4.45[a] | 0.98 | 0.009 |
| White Line Lesion Score | 5.37 | 4.39 | 0.56 | 0.023 | 5.43 | 4.93 | 4.28 | 0.68 | n.s. |

**Conclusions** These results show that both breed and nutritional/housing regimes influence the development of hoof lesions in dairy cows. The reduced levels of total lesions and white line lesions indicate potential breed differences in relation to predisposition to development of lameness. The increased levels of sole lesions in cattle on the "Grass" rather than the "Low" treatment merits further investigation of both nutritional effects and environmental aspects, for example the condition of laneways required to access pasture.

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# The effect of foot lesions on locomotion score and spine posture measured using computerised motion analysis in early lactation Holstein dairy cows

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**Introduction** Locomotion scoring is an important tool to detect dairy cow lameness. A number of locomotion scoring systems use the posture of the back to identify lame cows (score 3). For example Sprecher *et al* (1997) suggested the presence of an arched back standing and walking indicates lameness. Flower and Weary (2006) developed this system to score cows while walking where a score 3 cow has a more severely arched back than a score 2 cow. However to date no work has been published characterising the degree of arching of the spine observed in lame animals and its relationship with foot lesions. This is important in the early detection of lameness and could be incorporated into locomotion scoring systems to improve the objectivity. The aim of this study was to assess the effect of foot lesions on spine posture of Holstein dairy cows in early lactation by motion analysis.

**Materials and methods** Twenty five lactating Holstein dairy cows (6 primiparous and 18 multiparous (parity 2- 6), mean parity 2.6 ± 0.28) were recruited to the study 6 ± 0.7 days post partum. Markers (4cm diameter) were attached to the skin over the spinous processes T3, T7, L1, L4, the cranial end of the sacral vertebrae (SA) and on the tail head (TA). A digital video camera (Cannon PAL MV690) was placed 15m perpendicular to an alley which was 1.6m wide and the field of view was 4.5m. Filming followed morning milking. The images were digitised using Simi Motion Analysis Software (Postfach 1518, D-85705 Unterschleissheim). Posture was assessed at full support; where the limb is just seen to bear weight. The distance between each marker and the ground was measured. Three strides were analysed for each cow. Cows were locomotion scored using the method of Flower and Weary *(*2006) from the films. Lesions were assessed 8-10 weeks later according to the method described by Flower and Weary (2006). Differences between mean marker height as a percentage of wither height were tested by Analysis of Variance using Genstat (Version 8, Lawes Agricultural Trust). Differences between number of lesions was tested using a Fishers exact test.

**Results** There was no statistical effect of locomotion score on the height of any of the spine markers. However the degree of arching of the spine at full support appears to be greater for cows with a locomotion score of 2 and 3 compared with cows with a score of 1; defined as having a normal gait (Figure 1).

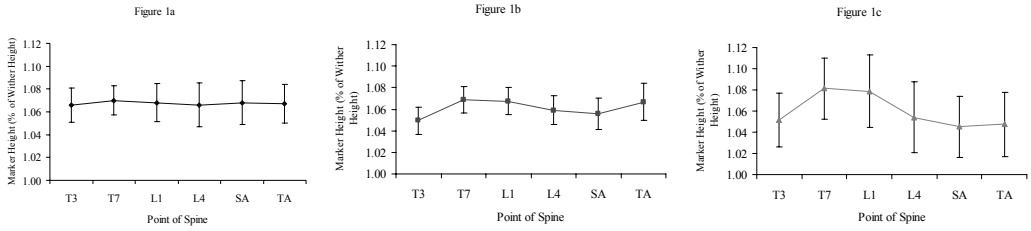


**Figure 1** Spine posture at full support of Holstein dairy cows in early lactation with a) locomotion score 1 n = 8, b) locomotion score 2 n = 11 and c) locomotion score 3 n = 5.

There were more ($p<0.05$) sole ulcers (SU) found on cows with a locomotion score of 3 compared to locomotion 1 (Table 1). There was at least 1 sole lesion on all the cows with a locomotion score 3. Cows with SU had significantly higher ($p<0.01$) locomotion score of 2.75 ± 0.25 compared to cows with haemorrhage (H) (1.92 ± 0.23) and those with no visible lesions (1.25 ± 0.25). Most (54%) haemorrhage occurred on the typical sole lesion site. Cows with locomotion score 3 had significantly higher ($p<0.01$) severity scores compared to cows with score 1 and 2.

**Table1** Number and type of hoof lesions measured 8 weeks after filming

| | Locomotion Score 1 (n= 6) | Locomotion Score 2 (n= 9) | Locomotion Score 3 (n= 5) |
|---|---|---|---|
| No Lesions | 3 | 2 | 0 |
| Haemorrhage (H) | 3 | 6 | 2 |
| Sole Ulcer (SU) | 0 | 1 | 3 |
| Lesion Severity | 0.67 | 0.89 | 2.40 |

**Conclusion** These results suggest that cows with severe lesions are more likely to have a higher degree of arching of the back than those with less severe lesions. In addition, they suggest that spine posture assessed using subjective and objective locomotion scoring systems has the potential to detect early the presence of sole ulcers and other hoof lesions. This hypothesis will be further tested in studies involving more animals.

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# Effects of level and form of dietary zinc on dairy cow performance and health

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**Introduction** It is well established that zinc is an essential micro-nutrient required to maintain health and performance in dairy cows (Underwood and Suttle, 2004). Cattle diets are traditionally supplemented with inorganic minerals (e.g. zinc oxide), but these may be poorly absorbed, resulting in an economic and environmental cost due to excess minerals being excreted. It is claimed organically bound minerals are able to resist interaction before and at the absorption site in the small intestine (Power, 2006), which may result in a lower dietary inclusion rate being required. The objective of the current experiment was to investigate the effect of an organically bound source of Zn as a replacement for inorganic Zn on dairy cow health and performance when supplemented at and below the recommended level.

**Materials and methods** A total mixed ration containing (DM basis) 0.39 maize silage, 0.19 grass silage, 0.08 urea treated wheat, 0.08 soyabean meal, 0.08 rapeseed meal , 0.07 molassed sugar beet pulp, 0.03 maize gluten feed, 0.05 molasses, 0.01 fat and 0.01 minerals (without zinc) was offered at 1.05 of *ad libitum* intake. The basal diet was predicted to supply 811mg of Zn per day and was supplemented with one of four concentrates differing in their level and form of dietary Zn. The concentrates provided an additional 600 mg Zn/day (to supply recommended levels (NRC, 2001); H) or 120 mg Zn/d (to supply 0.8 of the recommended level per day; L), either supplemented as ZnO (I) or organically bound Zn (Bioplex Zn$^{TM}$; Alltech Inc., Nicholasville, USA) and were fed at the rate of 2 kg/cow/day in one meal through out of parlour feeders. Forty-four Holstein-Friesian dairy cows (12 primiparous and 34 multiparous), that were on average 31 days (s.d. +/- 11.4) into lactation were allocated to one of the four treatments. Milk yield and feed intake were recorded daily and milk composition weekly. Additional milk samples were taken at the beginning of the experiment and then fortnightly for subsequent analysis of somatic cell counts (SCC) and milk amyloid A (Tridelta, Ireland). Animals were weighed and condition scored weekly. All cows remained on treatment for 14 weeks The data was analysed by analysis of variance as a 2 x 2 factorial design with treatment degrees of freedom split into main effects of Zn level (High *vs.* Low), form of Zn (organically bound *vs.* inorganic) and their interaction. All statistical analysis was conducted using GenStat version 8.1 (VSN Int. Ltd., Oxford, UK).

**Results** Cows supplemented with organically bound zinc at the recommended level of inclusion (HO) had a higher ($P<0.05$) milk yield than those fed inorganic zinc at the recommended level (HI) or organically bound zinc at the low level (LO). Dry matter intakes averaged 23.5 kg/d and did not differ ($P>0.05$) between treatments. Milk composition was not affected by treatment ($P>0.05$). Animals that received the low level of Zn (LI and LO) had higher SCC ($P<0.05$) and milk amyloid A levels ($P<0.05$) than those receiving the high levels (HO and HI). There was no effect ($P>0.05$) of treatment on body condition score or live weight.

**Table 1** Effect of level and form of dietary zinc on dairy cow performance and health.

| | Treatments | | | | | Significance† | | |
|---|---|---|---|---|---|---|---|---|
| | HI | HO | LI | LO | s.e.d | L | F | LxF |
| Total DM intake (kg/d) | 22.8 | 23.7 | 23.1 | 24.0 | 0.819 | 0.590 | 0.139 | 0.862 |
| Milk yield (kg/d) | 35.2$^{a}$ | 37.6$^{b}$ | 36.0$^{ab}$ | 35.2$^{a}$ | 0.96 | 0.268 | 0.247 | 0.026 |
| Fat (g/kg) | 40.7 | 41.0 | 41.9 | 42.0 | 1.67 | 0.372 | 0.909 | 0.879 |
| Fat yield (kg/d) | 1.44 | 1.50 | 1.49 | 1.50 | 0.053 | 0.312 | 0.525 | 0.557 |
| Protein (g/kg) | 33.2 | 32.4 | 33.8 | 33.0 | 1.04 | 0.418 | 0.274 | 0.995 |
| Protein yield (kg/d) | 1.16 | 1.19 | 1.21 | 1.15 | 0.065 | 0.967 | 0.754 | 0.388 |
| SCC (log (base e)) | 3.97 | 3.93 | 4.35 | 4.55 | 0.430 | 0.022 | 0.717 | 0.587 |
| Milk amyloid A (µg/ml) | 0.90 | 0.88 | 1.21 | 1.57 | 0.295 | 0.023 | 0.412 | 0.375 |
| Live weight (kg) | 609 | 608 | 621 | 616 | 10.1 | 0.114 | 0.707 | 0.803 |
| Condition score | 2.49 | 2.33 | 2.48 | 2.53 | 0.090 | 0.205 | 0.459 | 0.164 |

**Conclusions** Supplementation of Zn at the recommended level compared with below the recommended level reduced milk SCC and milk amyloid A, but there was no effect of form of supplementation. At the recommended level of inclusion supplementing Zn in an organically bound form increased milk yield by 2.4 kg/d, but there was no effect at the low level.

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# Herd factors affecting somatic cell count in Irish dairy herds

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**Introduction** The Irish milk payment system penalises against high milk somatic cell count (SCC). Previous studies have related farm management practices to herd SCC (Barkema *et al.,* 1998); however similar study has never been undertaken in Ireland. Furthermore, these previous studies have generally been conducted in confined systems. The objective of this study was to investigate potential management factors affecting herd SCC in Irish, spring calving, grass based dairy herds.

**Materials and methods** Annual milk supply data of farmers supplying to a major milk processor in Ireland were extracted and divided into strata in increments of 10,000 litres; so a representation of all farm sizes was achieved. A random sample from within each strata, weighted by the proportion of farmers in each stratum was taken, to provide a sample size of 398 herds. A total of 398 farmers were visited between April and July, 2006. A questionnaire consisting of 70 questions relating to pre-and post-milking practices, milking machine and milking parlour type, cleanliness of the facilities, and milk recording was applied during visit. Monthly test-day SCC data for the previous year were obtained. The average of the natural logarithm of SCC was calculated and this variable is referred to as somatic cell score (SCS). Data were analysed in PROC GLM (SAS, 2006) with SCS included as the dependent variable. Farms were categorised into 1 of 5 geographical regions for statistical analysis. Initially, a series of analyses including each potential independent variable was undertaken and factors that significantly affected SCS were retained for further analysis. A multiple regression model was developed using a stepwise regression approach on the variables that fulfilled the initial selection criteria. Diagnostics were undertaken on the residuals from the final model.

**Results** Herd size of the 398 participating farms ranged from 12 to 293. Somatic cell score varied from 11.32 SCS units (i.e. 82,207 SCC/ml) to 13.56 SCS units (i.e. 772,973 SCC/ml); median SCS was 12.53 units. After accounting for the geographical location of the farm, five factors significantly ($P<0.05$) affected SCS (Table 1).

**Table 1** Effect of management factors, significant ($P<0.05$) in the multiple regression model on SCS

| Management factors | SCS | SCC *1000 |
|---|---|---|
| Milking machine type | | |
| Direct pipeline | 12.65 | 31 |
| Recording pipeline | 12.53 | 28 |
| Heated water in the parlour | | |
| Yes | 12.54 | 28 |
| No | 12.64 | 31 |
| Cleanliness of parlour | | |
| Clean | 12.53 | 28 |
| Slightly dirty | 12.63 | 31 |
| Dirty | 12.61 | 30 |
| Using dry cow therapy | | |
| Yes | 12.49 | 27 |
| No | 12.69 | 32 |
| Milk recording | | |
| Yes | 12.56 | 28 |
| No | 12.62 | 30 |

**Conclusions** Five management factors significantly affected herd annual SCS with a lower SCS observed in herds that had either a recording plant pipeline, used heated water in the parlour, had a clean parlour, used dry cow therapy or milk recorded.

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## Effects of lamb sucking on the bacterial flora of teat duct and mammary gland of ewes

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**Introduction** It has been proposed that sucking by lambs is associated with transfer of microorganisms into the teat. There are three different sources of bacteria for the teat duct, namely the lamb (mouth, nasopharynx), the ewe (udder skin) or the environment (bedding). The bacteria can ascent to the mammary gland and cause mastitis. Objectives of the study were i) to determine differences in bacterial flora populations in the teat duct and mammary secretion of ewes before and after sucking and ii) to evaluate factors which affect these differences.

**Materials and methods** Eleven dairy Karagouniko-breed lactating ewes (7 with a single, 4 with twin lambs) were included in the study, carried out under a licence for experimental procedures. Paired-samples were obtained from both mammary glands of each ewe, on three occasions weekly, for six weeks (2nd to 7th week of lactation). Lambs were separated from their dams for 60 min; samples ("A") were collected and 30 s later, lambs joined their dams for sucking. New samples ("B") were collected with a different schedule on each of the three weekly sampling occasions. For samples "$B_1$" (1st sampling), natural termination (i.e., by the lamb or the ewe, without human interference) of a sucking bout was allowed; for samples "$B_2$" (2nd sampling), lambs were removed from their dam 3 min after joining her; for collection of samples "$B_3$" (3rd sampling), lambs were removed from their dam 1 min after joining her. Samples were obtained within 30 s. Further samples ("C") were collected 120 (±5) min after collection of "$B_1$". A sterile, plastic, 20 G catheter (Abbocath®) was used for sampling teat duct material (Mavrogianni *et al.*, 2006); this was followed by collection of milk samples. Standard methods for processing samples (aerobic culture) and bacterial identifications (biochemical tests, API-SYSTEM) were used (Mavrogianni *et al.*, 2005). Results were analysed by comparing changes in infection status between "A"-"B" and between "$B_1$"-"C", for duct material and secretion. Significance was assessed by the Sign Test. Furthermore, comparisons were performed in measurements from ewes with a single or with twin lambs, as well as between "$B_1$", $B_2$" and "$B_3$" samples. Finally, changes between stages of lactation (I: 2nd and 3rd week of lactation, II: 4th and 5th week, III: 6th and 7th week) were evaluated by using analysis of variance. Data were modelled in Minitab 14.

**Results** The bacteriological findings are in Table 1. After sucking, there was an increase of infected teat ducts by 200% ($P$<0.001), but no effect was evident for milk ($P$=0.590). Among the 40 duct material samples with a change of bacteriological status in-between sucking, 6 became negative, whilst 34 became positive. No significant differences were evident either among sampling procedures ("$B_1$", "$B_2$", "$B_3$"; $P$>0.540), or among ewes with a single or twins lambs ($P$=0.346), or among stages of lactation ($P$>0.420). Subsequently (120 min after sucking), there was a decrease of infected teat ducts by 31% (from 13 to 9) ($P$=0.375), but no change for milk. Most isolates were coagulase-negative staphylococci: 63/81 of total isolates; other organisms were: streptococci, *Escherichia coli*, *Bacillus* spp., *Mannheimia haemolytica* (2 isolates from duct material obtained after sucking), *Arcanobacterium pyogenes*, *Klebsiella* sp. and *S. aureus*.

**Table 1** Bacteriological status of samples from ewes before and after suckling of lambs, according to number of sucking lambs or stage of lactation (for description of stages, see text)

| | Ewes with singles | Ewes with twins | Stage I | Stage II | Stage III | Total |
|---|---|---|---|---|---|---|
| "A" Ducts | 5/252† | 9/144 | 3/132 | 5/132 | 6/132 | 14/396 |
| "A" Glands | 3/252 | 3/144 | 3/132 | 2/132 | 1/132 | 6/396 |
| "B" Ducts | 21/252 [19/21‡] | 21/144 [15/21] | 13/132 [11/13] | 13/132 [10/13] | 16/132 [13/16] | 42/396 [34/42 |
| "B" Glands | 4/252 [2/4] | 4/252 [1/4] | 3/132 [1/3] | 3/132 [1/3] | 2/132 [1/2] | 8/396 [3/8] |
| "C" Ducts | 5/84 [3/5] | 4/48 [1/4] | 2/44 [0/2] | 3/44 [2/3] | 4/44 [2/4] | 9/132 [4/9] |
| "C" Glands | 1/84 [1/1] | 1/48 [0/1] | 2/44 [1/2] | 0/44 [0/0] | 0/44 [0/0] | 2/132 [1/2] |

† n/m = bacteriologically positive results out of total samples; ‡ k/l = samples bacteriologically positive for the first time out of total bacteriologically positive samples

**Conclusions** The small rate of bacterial transmission is in accord with the generally small incidence of ovine mastitis. However, sucking clearly increases risk of infection of teat duct of ewes. Teats can withstand (indicated by minimal milk infections) and minimize (indicated by results of samples taken 120 min later) the infection. *M. haemolytica*, a confirmed mammary pathogen, was transmitted during sucking activity, from the tonsils of lambs.

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# Isolation of lactic acid bacteria from chickens that demonstrate probiotic properties of autoaggregation and coaggregation with Salmonella enteritidis.

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**Introduction** The association of salmonella infections with the consumption of poultry products and the fact that in the live bird the *Salmonella* carriage is mainly asyptomatic have led to a demand for finding ways of preventing infection of commercially reared poultry and product contamination (Revolledo *et al*., 2006). One approach is the use of probiotics. The probiotic properties of lactic acid bacteria have been widely studied. Their capacity for adhesion to mucus, ability to autoaggregate and potential for coaggregation with pathogenic bacteria are potential mechanisms for providing a competitive advantage in the intestinal microbiota (Ghadban *et al*, 2002) and forming a barrier that prevents colonization of pathogenic microorganisms (Kos *et al*, 2003). In this study, a total of 53 lactic acid bacteria (LAB) were isolated from the contents of the crop, caecum and small intestine, and from the mucosa of the crop, jejunum and ileum of three organically farmed chickens, were examined for autoaggregation and coaggregation with *Salmonella enteritidis*.

**Materials and methods** Autoaggregation was assessed according to the method of Kmet *et al* (1999). Briefly, 24h cultures of LAB (MRS - De Man, Rogosa, Sharpe broth, Oxoid, at 37$^{o}$C in 5% $CO_2$), were centrifuged (10 min at 10000xg), washed three times with sterile distilled water, resuspended in phosphate-buffered saline (PBS) at a concentration of $10^9$ CFU ml$^{-1}$ and incubated at room temperature in the presence of 10% (v/v) freshly prepared filter-sterilised own supernatant fluid. Autoaggregation was taken as positive when clearly visible sand-like particles (formed by the aggregated cells) gravitated to the bottom of the tubes, leaving a clear supernatant fluid, within 2h. The reaction times giving the degree of aggregation intensity, rapid: 15min (+++), normal: 15-30min (++) and slow: 30-60min (+). Coaggregation with *S. enteritidis* was assessed according to the method of Drago *et al* (1997). Briefly, 500µl of each LAB suspension ($10^9$ CFU ml$^{-1}$) were mixed with 500 µl of *S. enteritidis* ($10^9$ CFU ml$^{-1}$, human isolate supplied by the Public Health Laboratory Exeter) suspension and incubated in vials for 4 h at 37$^{o}$C under agitation. After 4 hours aggregation of the suspensions was scored as above and suspensions were prepared for observation by scanning electron microscope (JEOL 5600 Low Vacuum SEM). Six randomized fields were evaluated in each sample. LAB strains were identified using API CHL kit.

**Results** From 53 LAB that were tested for their capacity to aggregate, 20 were non-aggregative. Eleven bacteria showed a rapid autoaggregation, within 15minutes. 12 LAB had a normal reaction and the rest of the strains showed weak autoaggregation activity. The 23 LAB that showed normal and rapid aggregative activity were further tested for their ability to coaggregate with *S. enteritidis*. One LAB strain showed maximum aggregation (scored as 4, Fig. 1), two showed marked aggregation (3), six showed good aggregation (2), nine partial aggregation (1) and three showed no or almost no aggregation (0). Results were confirmed by scanning electron microscopy so that organisms with only autoaggregative properties were excluded from coaggregation scores (Drago *et al*, 1997). Interaction between the origin, the intensity of autoaggregative and the coaggregation with *S. eneritidis* could not be found.

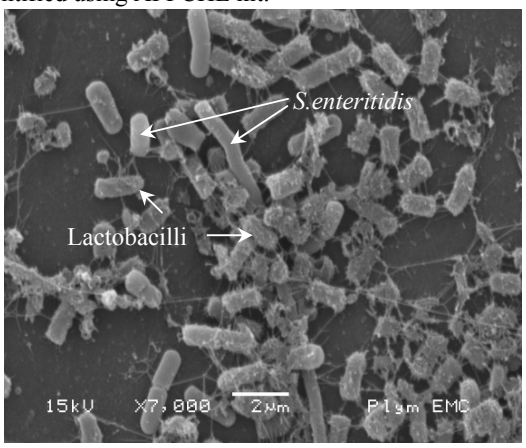


**Figure 1** Aggregation of a lactobacillus strain with *S.enteritidis* showing strands of LAB aggregating protein adhering to *S. enteritidis*

**Conclusions** It has been suggested that there is an association between the aggregation and epithelial adhesion ability of bacteria, which may contribute to the exclusion of pathogenic bacteria (Kos *et al*, 2003). Therefore the LAB strain that gave a rapid autoaggregation activity and a maximum ability to aggregate with *S. enteriridis* could be used for further screening for its potential use as probiotic in chicken nutrition.

**Acknowledgements** Financial support of the Greek State Scholarships Foundation is acknowledged.

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# The interaction between protein content and inulin inclusion on the health and performance of weaned pigs exposed to an enterotoxigenic Escherichia coli challenge

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**Introduction** Decreasing dietary crude protein (CP) supply has been shown to decrease the occurrence of post weaning colibacillosis (PWC) in pigs by reducing substrate availability to enterotoxigenic *Escherichia coli* (ETEC) (Prohászka and Baron, 1980). However, associated with the decreased risk of PWC is a detrimental effect on performance (Wellock et al., 2006). Non-starch polysaccharides (NSP), such as those derived from inulin, are a main energy source for microbial fermentation. Increasing inulin inclusion in weaner diets may therefore decrease bacterial need to ferment protein as an energy source and counteract the negative effects of protein fermentation, such as the production of harmful fermentation end products. Consequently, this may allow greater levels of protein to be included in the diet without compromising health. This in turn should allow greater levels of performance to be achieved. The aim was to investigate interactive effects of dietary CP supply and inulin inclusion on the health and performance of newly weaned pigs when artificially challenged with ETEC.

**Materials and methods** A 3 x 2 factorial design experiment compared dietary crude protein content (L; 150 vs M; 190 vs H; 230g CP/kg) and inulin inclusion (-, 0 g/kg vs +, 50 g/kg) over 2 experimental rounds. Sixty-six male pigs were weaned at 21.5 ± 0.81 days (± S.D.) of age and 6.9 ± 1.04 kg body weight, balanced for initial weight and litter and offered *ad libitum* access to one of the six dietary treatments. All diets were formulated to contain 16.0 MJ DE/kg and were balanced for amino acid composition as a proportion of total protein, sodium and lactose content. Pigs were challenged *per os* with $10^9$ ETEC (*E. coli* O149) on either day 4 or 7 post weaning (day 0). Feed intake, weight gain and faecal score (FS; assessed on a 4 point scale, where 1 = firm and 4 = watery) were recorded and fresh faecal samples were collected regularly to assess ETEC shedding. All animals were individually housed and euthanased on day 10 post infection for assessment of gut health and development. The effect of CP and inulin on the number of clinical PWC cases was determined by GLM using binomial proportions. Other analysis was performed using the REML procedure with infection day as a fixed effect. The animal experiment committee of SAC approved this work.

**Results** Table 1 shows the effect of treatment on pig health and performance. Fourteen animals were removed from the experiment prior to infection due to poor feed intake. In round one, 7 pigs suffered from clinical PWC post infection. The majority of these animals were from the M- and H- treatment groups. As a consequence, infection was delayed until day 7 post weaning in round 2, where none of the infected animals suffered clinical PWC. Decreasing CP level decreased ETEC shedding, although this was only significant on day 10 post-infection. Inulin inclusion did not affect ETEC shedding. Decreasing CP level decreased FS immediately post infection (P = 0.003). Pigs on the L diets gained less (P = 0.043) than those on the M and H diets with average daily gains (ADG) (± s.e.) of 106 (± 28.8), 215 (± 34.2) and 209 (± 34.8) g/d respectively over the -4 to 10 day period. There was no effect of CP level on average daily feed intake (ADFI) or feed conversion ratio (FCR). Inulin inclusion did not affect ADG, ADFI or FCR. There were no significant CP x inulin interactions.

**Table 1** The effect of crude protein level (L vs M vs H) and inulin inclusion (- vs +) on number of clinical cases of PWC, faecal score (FS), ETEC shedding and performance throughout the trial period

| | L- | L+ | M- | M+ | H- | H+ | s.e.d. | Response |
|---|---|---|---|---|---|---|---|---|
| Clinical cases of PWC | 0/7 | 1/8 | 3/8 | 1/9 | 2/10 | 0/10 | | |
| FS 24 hr post infection | 1.11 | 1.14 | 1.42 | 1.59 | 1.91 | 1.71 | 0.285 | CP** |
| ETEC days 0-10 ($log_{10}$ cfu/g) | 4.63 | 4.71 | 5.39 | 4.80 | 5.06 | 5.85 | 0.991 | |
| ADFI days -4 to 10 (g/d) | 150 | 244 | 223 | 285 | 290 | 257 | 47.6 | |
| ADG days -4 to 10 (g/d) | 57 | 161 | 180 | 245 | 234 | 187 | 47.5 | CP* |
| FCR days -4 to 10 | 2.46 | 1.80 | 1.87 | 1.00 | 1.20 | 1.33 | 0.707 | |

Main effects * P < 0.05, **, P < 0.01; where CP = Crude protein level

**Conclusion** Decreasing dietary CP level improved pig health but decreased performance. The addition of inulin had no significant effects on pig health and performance and the hypothesised interaction between protein and inulin was not observed. The relatively high incidence of clinical PWC and the consequent removal of pigs from the experiment may account, at least in part, for the lack of significant results. The fewer cases of clinical PWC observed in pigs fed the inulin diets (P = 0.164) warrant further investigation.

**Acknowledgements** This research was financially supported by ABNA Ltd, Frank Wright Ltd, Home-Grown Cereals Authority, Meat and Livestock Commission/British Pig Executive, Primary Diets Ltd and Provimi Ltd with match funding from Defra, through the Sustainable Livestock Production LINK programme.

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# An investigation into the level of fusarium mycotoxins in samples of UK wheat straw used for bedding livestock

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**Introduction** Straw based production systems are common in the UK compared to European, Asian and American competitors. The effects of mycotoxins in cereal feed on livestock performance are relatively well documented with pigs being particularly sensitive to mycotoxicosis. The European Commission has recently set guideline limits for fusarium mycotoxins in animal feedstuffs (Anon, 2006). Guidance limits for feedstuffs for young pigs is 900 ppb deoxynivalenol and 100 ppb zearalenone. However, any possible effects of ingestion of fusarium mycotoxins from bedding have not been enumerated. This project was designed to evaluate the potential risk of mycotoxin challenge from straw bedding in the UK.

**Material and methods** A total of 40 samples of wheat straw were collected from 31 farms across eight counties in the midlands and south of England. All straw samples were from the 2005 harvest and collected in July 2006. Each sample was collected from several points in the interior of a single bale. Data was collected detailing the variety, agronomy, bale type and conditions of harvest and storage. There were no overtly mouldy samples taken and the samples were scored on a five point scale for colour/condition (1-Clean, weed free, minimal dust, 5- Very dusty, with large proportion of dark stems and weeds). Each sample was dried to ca. 12% moisture content and carefully sub-sampled. Sub-samples of ca. 100 g were ground in a hammer mill with a 1mm screen. Zearalenone (ZEAR) and deoxynivalenol (DON) were analysed using Ridascreen DON and ZEAR ELISA test kits, which based on the modified extraction procedure had limits of quantification for DON and ZEAR of 75 and 7 ppb respectively. Data were normalised using a log transformation and analysed using Genstat 8.1.

**Results** There were no significant correlations between ZEAR, DON and the sample colour/condition score and there was no effect of bale type, storage or production method on the concentration of ZEAR and DON. The concentrations of fusarium mycotoxins in wheat straw (Table 1) were higher than that found in wheat grain, which had a median of <5 and 65 ppb for zearalenone and DON respectively in 2005 (SG Edwards, unpublished data), with a similar skewed distribution. One sample exceeded the zearalenone guidance limit for pig feed (2.5%) and five samples (12.5%) exceeded the DON guidance limit for pig feed.

**Table 1** Concentration of fusarium mycotoxins in UK wheat straw

| | Mycotoxin concentration (ppb) | |
|---|---|---|
| | Zearalenone | Deoxynivalenol |
| Mean | 23 | 460 |
| Median | 9 | 284 |
| 95th Percentile | 34 | 1343 |
| Maximum | 498 | 1955 |

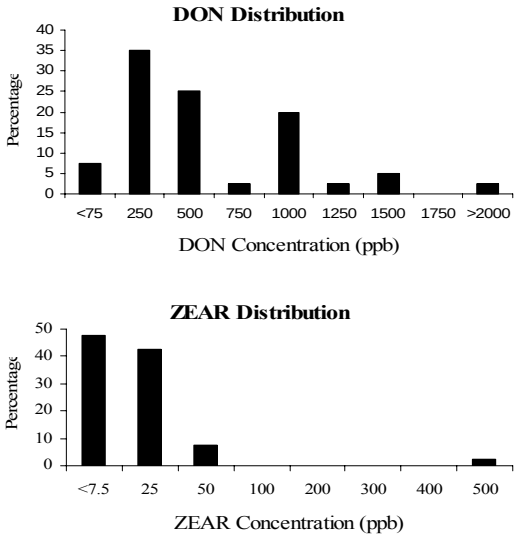


**Figure 1** Percentage frequency distribution of fusarium mycotoxin concentrations in UK wheat straw samples.

**Conclusions** UK wheat straw can contain significant levels of fusarium mycotoxins. Straw consumption by weaner pigs on deep bedded straw is estimated at between 7.4 and 13.1 %TDI (van Barneveld, 2005) however intake by dry sows is likely to be >2kg/d. Based on the levels of fusarium mycotoxins found in UK straw then straw could be a significant proportion of the mycotoxin load consumed by pigs and contribute to sub-clinical (reduced weight gain) and clinical mycotoxicosis. Farmers using a straw based bedding system should consider straw as a component of the diet and as such it should be tested as part of any investigation of mycotoxicosis.

**Acknowledgements** This work was undertaken as a summer studentship by Claire White. Financial support of British Society of Animal Science, British Pig Executive and Harper Adams University College is gratefully acknowledged. We would also like to thank the commercial producers that kindly supplied the samples.

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# The incidence of calf mortality on dairy farms in southern England

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**Introduction** Reduced reproductive performance of dairy cows is a major challenge facing the dairy industry. Many potential replacement heifers fail to reach their first lactation because they are either born dead, die as juveniles, or fail to conceive. Perinatal mortality has previously been estimated across English herds as 7.8% (Esslemont & Kossaibati, 1996), whereas neonatal mortality in the UK has been estimated at 0 to 10% (reviewed by Mellor & Stafford, 2004). Calf mortality is often poorly documented and estimates that are available are possibly underestimated because a dead calf at birth is not always recorded at the farm level. Therefore, the objective of this study was to accurately calculate the incidence of perinatal and neonatal mortality of dairy calves on commercial farms in southern England.

**Materials and methods** A total of 18 dairy farms milking Holstein-Friesian cows were recruited between September 2003 and October 2004. These provided a range of management practices representative of those commonly encountered in the UK. Over a time period of approximately 2 months per farm, details on all calves (male and female) born alive, as well as those born dead or that died during the first month of life were recorded. Each farm was visited to check records regarding every birth (dead or alive) during the time period selected, thus ensuring the accuracy of the data. Using this dataset of 1097 records, perinatal mortality (defined as stillbirths and mortality of males and females, during the first 24 hours of life) and neonatal mortality (defined as female calves born alive that die between 24 hours and 28 days) was calculated on the individual farms. Perinatal mortality in this study included both males and females because some farms did not record the sex of calves born dead.

**Results** The average perinatal mortality across 18 farms was 8.1%, ranging from 3% to 14% on individual farms, and neonatal mortality averaged at 3.4%, ranging from 0% to 13% on individual farms (Table 1). There was a trend for neonatal mortality to increase with increasing herd size (2.2% <200 cows, 4.9% >400 cows), and more calves died during autumn and winter (6.2%) compared with spring and summer (2.6%) ($P$<0.05).

**Table 1** Calf mortality (perinatal mortality of males and females, and neonatal mortality of females)

| Farm | Herd size | Calving season | No. of calves born | % of calves born dead (n) | No. of females born alive | % of females dying in 1st month (n) |
|---|---|---|---|---|---|---|
| 1 | 200-400 | Summer | 65 | 8 (5) | 32 | 3 (1) |
| 2 | <200 | All year | 43 | 5 (2) | 19 | 11 (2) |
| 3 | <200 | Summer | 64 | 6 (4) | 28 | 0 |
| 4 | >400 | Autumn | 180 | 7 (12) | 78 | 4 (3) |
| 5 | 200-400 | Winter | 53 | 9 (5) | 24 | 0 |
| 6 | >400 | Spring | 42 | 5 (2) | 24 | 4 (1) |
| 7 | 200-400 | Autumn | 73 | 3 (2) | 30 | 0 |
| 8 | <200 | Autumn | 57 | 12 (7) | 27 | 0 |
| 9 | >400 | Autumn | 65 | 5 (3) | Unconfirmed | |
| 10 | 200-400 | Summer | 62 | 11 (7) | 27 | 4 (1) |
| 11 | 200-400 | Summer | 80 | 11 (9) | 33 | 12 (4) |
| 12 | <200 | Autumn | 45 | 4 (2) | 26 | 4 (1) |
| 13 | <200 | Autumn | 42 | 14 (6) | 21 | 0 |
| 14 | <200 | Summer | 37 | 11 (4) | 20 | 0 |
| 15 | <200 | Summer | 50 | 4 (2) | 25 | 4 (1) |
| 16 | >400 | Spring | 57 | 14 (8) | 31 | 13 (4) |
| 17 | >400 | Spring | 48 | 8 (4) | 29 | 0 |
| 18 | <200 | Autumn | 34 | 9 (3) | 20 | 0 |
| Mean | | | | 8.1% | | 3.4% |

**Conclusions** Under good management conditions, it has been suggested that perinatal mortality can be maintained at 1 to 3%, and neonatal mortality at 3% (Heinrichs & Radostits, 2001). This study has demonstrated that perinatal mortality rates in the UK continue to be considerably higher than this suggested target. Furthermore, the percentage of animals born dead in this study is the same as that estimated by Esslemont and Kossaibati (1996), demonstrating that perinatal mortality rates have changed very little in the past 10 years. Mortality at birth of 8%, together with a further loss of 3% during the first month of life represents a large loss of potential replacement dairy heifers. Systems need to be in place on farms to monitor the incidence of calf mortality, with the aim of reducing it by the introduction of improved management practices.

**Acknowledgements** We are grateful to DEFRA and the Milk Development Council (MDC) for financial support.

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# Evaluation of Video Image Analysis (VIA) technology to predict meat yield of sheep carcasses online under abattoir conditions

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**Introduction** Accurate estimates of carcass composition and eating quality are critical to the introduction and the success of a value-based marketing system (VBMS) and to help address increased consumer demands for leaner meat with higher quality. Currently in the UK, carcass composition is assessed by a subjective carcass classification system based on the EUROP conformation system, and a visual assessment of fat cover using a numeric fat score ("MLC Scoring") (Anderson, 2003). Objective, image analysis based systems to classify carcasses into current classification categories have been studied (Allen and Finnery, 2000) and are in use in the beef industry in the EU. However, the introduction of automatic technologies such as VIA may also have considerable potential for prediction of lean meat yield of the carcass. There is growing interest in the possibility of developing payment criteria which are based on carcass meat yield. Therefore, the present research project investigated the potential of VIA technology to predict meat yield in terms of saleable meat yield (SMY), saleable primal meat yield (SPMY) and the carcass components leg, chump, loin and shoulder in lamb.

**Materials and methods** A data set of 295 lamb carcasses was used to calibrate the E+V VSS2000 VIA system in the prediction of meat yield. Carcasses were dissected at Welsh Country Foods (WCF) using standardised butchery specifications. The whole carcass cold weight (CCW) was recorded and the carcasses were separated into three opening primal cuts - fore/shoulders, saddle and legs. Weights of the three primal cuts (opening primals) were recorded and then they were separated into sub-primal cuts and the residuals of the trimming process lean tissue, fat and bone/waste under a generic butchery specification. Total SMY was the sum of weights of all sub-primal cuts plus the residual lean tissue of the trimming process as a proportion of CCW and SPMY was the sum of weights of all sub-primal cuts as a proportion of CCW. Linear least squares analysis (GLM of SAS) was used to estimate the coefficient of determination ($R^2$) and the root mean square error (RMSE)

**Results** Table 1 shows the $R^2$ and the RMSE of prediction of SMY, SPMY and kilograms of opening primals cuts leg-chump, loin-breast and shoulder by using VIA and MLC standard scores (fat and conformation class). The $R^2$ of prediction of SMY, SPMY, opening primal cuts of leg-chump, loin-breast and shoulder were 20, 17, 64, 44, and 67%, respectively, higher using VIA than using MLC standard score. The precision measured as reduction in RMSE of predicting SMY, SPMY, kilograms of leg-chump, loin-breast and shoulder were 13, 12, 85, 66 and 79%, respectively, higher using VIA than using MLC standard score. The effect of CCW, sex and slaughter day was tested and found significant in both MLC and VIA models ($P<0.05$). After adjustment of meat yield traits for differences in CCW, sex and changes between slaughter days, the precision measured as reduction in RMSE of predicting SMY, SPMY, opening primals cuts leg-chump and loin-breast increased by 6, 11, 28, 9 and 8% respectively using VIA, but a much larger increase of 13, 12, 85, 66, and 79% respectively, was obtained using MLC scores.

**Table 1**. Coefficients of determination ($R^2$) and root mean square errors (RMSE) of prediction of SMY, SPMY and carcass components using VIA traits or MLC standard scores, n=295

| | MLC standard | | | | VIA trait | | | |
|---|---|---|---|---|---|---|---|---|
| | Un-ajusted | | adjusted † | | Un-ajusted | | adjusted † | |
| Variables | $R^2$ | RMSE | $R^2$ | RMSE | $R^2$ | RMSE | $R^2$ | RMSE |
| SMY, % | 55.1 | 1.46 | 65.3 | 1.32 | 68.7 | 1.24 | 68.7 | 1.24 |
| SPMY, % | 53.9 | 1.37 | 66.9 | 1.20 | 64.6 | 1.20 | 72.9 | 1.07 |
| Openning Primals | | | | | | | | |
| Leg & chump, kg | 36.0 | 1.106 | 97.3 | 0.231 | 98.5 | 0.169 | 98.6 | 0.167 |
| loin & Breast, kg | 52.5 | 0.862 | 94.5 | 0.300 | 94.4 | 0.291 | 95.3 | 0.273 |
| Shoulder, kg | 31.9 | 1.311 | 97.2 | 0.273 | 96.9 | 0.273 | 97.5 | 0.251 |

† After adjustment for the effects of CCW, sex and slaughter date

**Conclusions** The precision of predicting total SMY, SPMY and their components using VIA were substantially higher than using MLC standard scores. In particular, lean meat of the entire body and its components could be estimated more accurately using VIA than using MLC standard scores. The adjustment of the prediction for the effects of CCW, sex and slaughter day increased the prediction's of both methods, especially the one based on the MLC scores, indicating that this prediction method is highly influenced by the differences in CCW, sex and the changes among slaughter days. In conclusion, the VIA system has been shown to be an accurate measurement technique for the prediction of carcass lean meat yield of sheep.

**Acknowledgements** Financial support of EBLEX, Hybu Cig Cymru, Quality Meat Scotland, Livestock and Meat Commission (Northern Ireland) and Genesis Faraday is gratefully acknowledged.

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# Influence of grass and concentrate feeding systems on lipid and colour shelf life of loin steaks from Charolais steers

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**Introduction** Diet has a large impact on the fatty acid composition of muscle lipids in cattle (Scollan et al., 2006). Grass relative to concentrate feeding increases the content of n-3 polyunsaturated fatty acids (PUFA) resulting in a low n-6:n-3 PUFA ratio and also increases the concentration of the antioxidant, vitamin E, in the meat. Ruminally protected plant lipids enhance PUFA content very significantly resulting in beneficial P:S and n-6:n-3 ratios, but without extra antioxidant input can lead to lipid oxidation and reduced colour shelf life. This study considered the effects of finishing steers (1) outdoors on grass ± concentrate versus (2) indoors on straw/concentrate ± a protected lipid supplemental with one of two levels of vitamin E on the sensory quality and lipid and colour stability of the loin muscle.

**Materials and methods** Forty eight Charolais steers (initial live weight 506 kg (s.e.d. 4.7)) were randomly allocated to one of six dietary treatments (each consisting of eight animals) (1) *ad libitum* grazed perennial ryegrass (G), (2) grazed ryegrass plus 2.5 kg concentrate (GC1), (3) grazed ryegrass plus 5 kg concentrate (GC2), (4) 80:20 (DM basis) straw + concentrate control (Control) (5) straw + concentrate (standard vitamin E, 25 mg/kg)+ 600 g/d protected lipid supplement (PLS; *n*-6:*n*-3 ratio of 1:1) (PLSV1) and (6) straw + concentrate (high vitamin E, 500 mg/kg DM) + 600 g/d PLS (PLSV2). Straw was offered *ad libitum*. The grazed animals were maintained on rotational grazed paddocks while the straw/concentrate treatments (4, 5 & 6) were kept indoors. All animals were fed to achieve a similar rate of carcass gain by either restricting grass (treatments 2, 3) or restricting intake of the concentrate. The concentrate was based on barley, molasses, sugarbeet pulp, megalac and premix. Animals were slaughtered after 100 days on treatment and samples of m. *longissimus thoracis et lumborum* (LTL) were taken at 48h post-mortem for vitamin E analysis and 10 day aged samples for sensory analysis, or shelf life studies in modified atmosphere packs. An ANOVA with diet as the main factor was used to analyse the data.

**Results** As the amount of concentrates in the diet increased so the amount of vitamin E decreased, being almost replenished to that of grass-grazed animals by dietary addition in PLSV2. Reduced vitamin E led to an increased oxidation of lipids as shown by the TBARS values, particularly so by 10 days of retail display and when additional PUFA were incorporated into the meat (see Scollan et al., 2007) when PLSV1 was fed, but the additional vitamin E in PLSV2 failed to produce the same meat stability as that for grass-grazed beef. Colour stability showed similar trends, though differences between diets were small and not statistically significantly different. The carotene content of the subcutaneous adipose tissue over the 10$^{th}$ rib was highest in grass fed animals. That found in the indoor-fed animals may have been residual from the prior dietary regime and appeared to be reduced by the additional vitamin E in PLSV2. Carotene concentration was linearly related to the b* (yellowness) of the fat.

**Table 1** TBARS (mg/100g muscle) and colour saturation of loin steaks during simulated retail display, vitamin E content (mg/100g) of muscle and carotene (mg/100g) content of adipose fat

| | Outdoor | | | Indoor | | | | |
|---|---|---|---|---|---|---|---|---|
| | Grass (G) | GC1 | GC2 | Control | PLSV1 | PLSV2 | SED | P |
| mg/100g lean tissue | | | | | | | | |
| Vitamin E | 4.51$^{c}$ | 3.86$^{b}$ | 3.85$^{b}$ | 2.72$^{a}$ | 2.81$^{a}$ | 4.04$^{b}$ | 0.40 | *** |
| TBARS d5 § | 0.36$^{a}$ | 0.40$^{a}$ | 0.62$^{a}$ | 1.34$^{b}$ | 2.07$^{c}$ | 0.49$^{a}$ | 0.297 | *** |
| TBARS d10§ | 0.66$^{a}$ | 0.95$^{a}$ | 1.45$^{ab}$ | 2.68$^{b}$ | 5.70$^{c}$ | 1.59$^{ab}$ | 0.815 | *** |
| Carotenes in adipose tissue | 0.65$^{c}$ | 0.57$^{bc}$ | 0.47$^{bc}$ | 0.39$^{ab}$ | 0.40$^{ab}$ | 0.25$^{a}$ | 0.086 | *** |
| Colour saturation day 8§ | 17.8 | 18.0 | 17.5 | 17.8 | 16.3 | 17.9 | 0.91 | NS |

§ days of retail display

The only difference found by the sensory panel was that the group fed grass and low concentrates produced meat which was significantly ($p<0.05$) tougher than the rest.

**Conclusions** Grass grazing not only produces meat with an improved n-3 fatty acid content but also produces more yellow fat due to its carotene content and a greater lipid stability due to the natural intake of vitamin E with the diet. Improving the P:S ratio by feeding protected lipids causes a severe oxidative challenge in the meat and this is overcome to a great extent by supplementing the diet with vitamin E. Supplementing animals with concentrates whilst grazing over a 100d period reduces the natural vitamin E content of the meat with a trend towards reducing its stability

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Scollan, N.G., Hallett, K.G., Wood, J.D. and Richardson, R.I. (2007) this proceedings

# Meat quality and carcass characteristics of Norwegian dairy and Holstein-Friesian cattle

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**Introduction** Any evaluation of breeds or production systems for beef must consider effects on production, carcass and meat quality characteristics. Holstein-Friesian (HF) cattle are bred for dairy traits only, while Norwegian dairy cattle (NOR) have been selected with some emphasis on beef characteristics. A comparison of production data from bulls of these two breeds has been presented previously (Kirkland *et al.,* 2005). The objective of the present study was to evaluate specific carcass and meat quality parameters of HF and NOR bulls.

**Materials and methods** A total of 64 bulls (33 HF, 31 NOR), mean initial age 177 (SD 43.3) days and live weight 194 (SD 63.9) kg were used in a 2 x 2 factorial design study encompassing 2 breeds and 2 slaughter ages. Cattle were penned, within breed, in groups of three animals. The two slaughter ages were 16 (E) and 20 (L) months, with animals being blocked, within breed, prior to the initial slaughter date according to similarity of age. One animal from each block was allocated at random to each slaughter age treatment group. Diets consisted of a 50:50 mix of grass silage and concentrates (dry matter basis). The composition of the concentrates was changed when animals reached 350 kg live weight and contained the following ingredients (g/kg) : barley 550 and 380, soya bean meal 175 and 145, sugar beet pulp 150 and 150, maize meal 100 and 300, vitamin/mineral premix 25 and 25 for concentrates offered pre- and post-350 kg live weight, respectively. Data on a range of carcass parameters were recorded from all animals post slaughter. The *longissimus dorsi* muscle from the fore-rib joint was removed 48 h post-mortem and two, 50 mm thick slices of the muscle were vacuum packed and aged for 7 days post-mortem at 2°C. Assessments of ultimate pH, colour, Warner Braztler shear force (WBSF) and cooking loss were undertaken using the methods detailed by Moss *et al*. (1993). Data were analysed using the REML technique in Genstat 5 (release 4.1 Rothamsted, England). Live weight and age at the start of the study (within breed), and age at slaughter (within slaughter age group), were incorporated into the analyses of carcass data as covariates. Data on meat quality were analysed without covariate adjustment.

**Results** Data on carcass characteristics and meat quality are presented in Table 1. No breed x slaughter group interactions ($P>0.05$) were recorded for any of the parameters evaluated in the study. Breed of bull had no ($P>0.05$) effect on depth of subcutaneous fat, marbling score, eye muscle area or on the weight of fat in the internal depots. However, HF bulls had lower L* ($P<0.05$) and WBSF ($P<0.001$) values, but had higher ($P<0.05$) $pH_u$ than NOR bulls. In contrast, a*, b*, chroma, $h^0$, and cooking loss were similar ($P>0.05$) between the breeds. Bulls in the L group had greater subcutaneous fat, marbling score, internal fat depots and eye muscle area ($P<0.01$ or greater) than those in the E group. Meat from bulls slaughtered in the L group had higher a* ($P<0.001$) and chroma ($P<0.01$) values, but lower $h^0$ ($P<0.01$) and cooking loss ($P<0.01$) values than those slaughtered in the E group. However, L*, b*, $pH_u$ and WBSF values were not significantly affected by age at slaughter.

**Table 1** The effect of breed and age at slaughter on carcass characteristics and meat quality

| | | Breed [1] | | | | Slaughter group [2] | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | HF | NOR | SED | Sig | E | L | SED | Sig |
| Carcass parameters | | | | | | | | | |
| Subcutaneous fat (mm) | | 3.7 | 3.1 | 0.32 | NS | 2.4 | 4.4 | 0.32 | *** |
| Marbling score [3] | | 2.6 | 2.2 | 0.18 | P=0.06 | 2.2 | 2.7 | 0.18 | ** |
| KCC fat (kg) [4] | | 17.4 | 15.9 | 1.35 | NS | 12.7 | 20.7 | 1.43 | *** |
| O & M fat (kg) [5] | | 28.9 | 28.4 | 1.91 | NS | 22.9 | 34.4 | 2.14 | *** |
| Eye muscle area ($cm^2$) | | 58.9 | 58.3 | 2.24 | NS | 55.6 | 61.6 | 2.25 | ** |
| Meat quality | | | | | | | | | |
| | L* | 34.5 | 37.3 | 1.15 | * | 35.3 | 36.5 | 1.15 | NS |
| | a* | 18.5 | 19.2 | 0.91 | NS | 17.2 | 20.5 | 0.91 | *** |
| Colour | b* | 12.4 | 13.3 | 0.84 | NS | 12.2 | 13.5 | 0.84 | NS |
| | Chroma | 22.3 | 23.4 | 1.18 | NS | 21.1 | 24.6 | 1.18 | ** |
| | Hue angle ($h^0$) | 33.7 | 34.2 | 0.99 | NS | 35.2 | 32.6 | 0.99 | ** |
| Ultimate pH | | 6.04 | 5.80 | 0.108 | * | 5.92 | 5.91 | 0.109 | NS |
| Cooking loss (%) | | 21.0 | 22.9 | 1.46 | NS | 24.2 | 19.7 | 1.46 | ** |
| 7-day WBSF ($kg/cm^2$) | | 2.40 | 3.03 | 0.175 | *** | 2.74 | 2.70 | 0.175 | NS |

[1] HF = Holstein-Friesian, NOR = Norwegian dairy breed; [2] Animals slaughtered at 485 (E) or 610 (L) days; [3] 8 point scale: 1 = low marbling; 8 = high marbling; [4] Kidney, cod and channel fat; [5] Omental and mesenteric fat

**Conclusions** The results of the present study indicate that HF and NOR bulls deposit fat in the internal depots to a similar extent. However, beef from HF bulls is more tender but a greater proportion of joints from this breed are likely to appear dark to consumers. Meat from both breeds was acceptably tender. Slaughtering bulls at 20 compared to 16 months of age increased fat deposition in the internal depots and improved colour characteristics.

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# Breed and sex effects on muscle fibre characteristics in two contrasting sheep breeds: Scottish Blackface and Texel

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**Introduction** Lamb production is an important part of UK agriculture, contributing more than 10% of total livestock output. It is crucial for maintaining employment and infrastructure in rural communities, and in managing and enhancing the countryside and biodiversity. For the UK sheep industry to continue as a major producer and exporter of lamb, the improvement of its economic sustainability is essential and requires high product quality.
As one of the major terminal sire breeds in the UK, Texel sheep (TEX) have been selected over generations for rapid growth, muscularity and lean meat content. In contrast, Scottish Blackface sheep (SBF) have been selected for their hardiness and maternal ability, with less attention being paid to carcass traits. It is clear therefore that divergent selection goals are driving the breeding of TEX and SBF. Based on work in pigs in particular, where selection for lean tissue growth rate has been used extensively, it might be predicted that these two breeds would have diver-gent muscle fibre characteristics. The proportion of muscle fibre types and their size affect muscularity as well as functional properties of the musculature and meat eating quality (MEQ). Breed differences indicate a genetic basis for these traits. This study evaluates the effects of breed and sex, together with those of birthweight and litter size on fibre type characteristics.

**Materials and methods** Entire male (M) and female (F) Texel (TEX, n=236) and Scottish Blackface (SBF, n=231) lambs, born in 2003 and 2004, were grazed together on lowland pastures in mixed-breed groups from birth to slaughter. Lambs were finished according commercial slaughter criteria, based on condition score and live weight, in 5 (2003) and 6 (2004) batches. Age at finishing ranged from 91 to 202 days, averaging at 139d. All lambs were scanned using Computed tomography (CT) at finishing point (FP) to assess loin muscle area and its CT- pixel density (LD), and then half of the lambs in each finishing batch were slaughtered. The other half were slaughtered 30d later to allow a withdrawal period for the CT sedative, resulting in 2 slaughter groups (SG). The fibre type characteristics of M. *longissimus dorsi* (LD) at the 12-13th rib position were determined through the use of immunohistochemical techniques based on 200 to 400 fibres as described earlier (Chang et al., 2003). Data were analysed using the GLM procedure (SAS 9.1) including breed, year, SG and sex as fixed effects, and live weights at birth and at FP as co-variates in the model.

**Results** Breed and, to a slightly lesser extent, sex affected almost all measured traits significantly (effects not shown) causing significant differences between the breed by sex groups (Table 1). Although data were adjusted for live weight, the LD of TEX, compared to SBF, had significantly higher total muscle areas (16%), higher total fibre numbers (20%) and higher muscle densities (5%). The frequency of slow fibres averaged 8.5% in both breeds and was much lower than that of fast fibres (91.3%). SF of TEX had, compared with SBF, a lower frequency and the area they occupied in the LD smaller (7.5% *vs.* 9.6% in SBF ). The results for FF showed the opposite situation: higher frequency and a higher relative area in TEX. The metabolic profile showed, with two different staining methods (SDH-Succinate dehydrogenase, SBL- Sudan black) a 10% lower frequency for oxidative fibres in TEX than SBF lambs. The inter fibre lipid content in TEX was significantly lower than in SBF

| | *TEX M* | *TEX F* | *SBF M* | *SBF F* |
|---|---|---|---|---|
| Slow fibres | | | | |
| Frequency (%) | 8.33 $^{ab}$ | 7.55 $^{b}$ | 9.21 $^{a}$ | 8.71 $^{a}$ |
| Area ($\mu m^2$) | 1123 $^{b}$ | 1107 $^{b}$ | 1227 $^{a}$ | 1258 $^{a}$ |
| % Area | 8.02 $^{b}$ | 6.91 $^{c}$ | 10.25 $^{a}$ | 9.02 $^{b}$ |
| Fast Fibres | | | | |
| Frequency (%) | 91.25$^{ab}$ | 92.15$^{a}$ | 90.69 $^{b}$ | 91.15 $^{b}$ |
| Area ($\mu m^2$) | 1198$^{a}$ | 1219$^{a}$ | 1091 $^{b}$ | 1228 $^{a}$ |
| % Area | 91.98$^{ab}$ | 93.09 $^{a}$ | 89.75 $^{b}$ | 90.98 $^{b}$ |
| Metabolic Profile | | | | |
| SDH+ve Frequency (%) | 52.78 $^{b}$ | 51.93 $^{b}$ | 61.88 $^{a}$ | 62.61 $^{a}$ |
| SDH+ve Area ($\mu m^2$) | 1143 $^{a}$ | 1121 $^{ab}$ | 1064 $^{b}$ | 1161 $^{a}$ |
| SBL+ve Frequency (%) | 45.24 $^{b}$ | 43.96 $^{b}$ | 54.31 $^{a}$ | 53.99 $^{a}$ |
| SBL+ve Area ($\mu m^2$) | 1122 $^{ab}$ | 1099 $^{ab}$ | 1061 $^{b}$ | 1156 $^{a}$ |
| Inter fibre lipid (% total area) | 1.94 $^{c}$ | 2.16 $^{bc}$ | 2.71 $^{b}$ | 3.24 $^{ab}$ |
| Muscle fibre parameters | | | | |
| LD cross-sectional area ($mm^2$) | 1302$^{b}$ | 1365$^{a}$ | 1112$^{d}$ | 1191$^{c}$ |
| Grand mean fibre area ($\mu m^2$) | 1160$^{b}$ | 1163 $^{b}$ | 1159 $^{b}$ | 1243 $^{a}$ |
| Total fibre no x$10^4$ | 1174 $^{a}$ | 1218 $^{a}$ | 998 $^{b}$ | 994 $^{b}$ |
| LD average pixel density | 57.04 $^{a}$ | 56.56 $^{a}$ | 54.49 $^{b}$ | 53.4 $^{c}$ |

**Table 1** Least squares means of muscle fibre traits for breeds and sexes. Breed by sex group means sharing a common character in their superscript are not significantly different (P>0.05).

**Conclusions** The presented breed differences in muscle fibre traits indicate underlying genetic variation and future analyses will evaluate the link of these traits to meat quality traits and estimate intra-breed heritabilities to assess the usefulness of these traits in breeding programmes. If these traits are sufficiently heritable and correlated to meat eating quality (MEQ) traits they could serve as possible selection traits or indicators for MEQ.

**Acknowledgements** Financial support of Defra and SEERAD is gratefully acknowledged.

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# The effect of recombinant caspase 3 on porcine myofibrillar proteins

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**Introduction** Meat tenderisation results from the weakening of the myofibrillar structures and has been attributed to endogenous proteolytic enzymes. It has been proposed that tenderization is a mulitenzymatic system and the process of slaughter and exsanguination would engage muscle cells in a form of cell death (Ouali *et al*., 2006). Caspases are primarily associated with apoptosis and once activated they target and cleave a number of substrates including components of the Z-disk and costameres. Recent studies have shown that caspases are active in skeletal muscle during the postmortem conditioning period and our preliminary data indicates that there is a relationship between caspase activity and shear force (Kemp *et al*., 2006). The aim of this study was to investigate whether recombinant caspase 3 was capable of porcine degrading myofibrillar proteins *in vitro*.

**Material and methods** Myofibrils were prepared from porcine *longissimus dorsi* (LD) muscle according to Goll *et al*. (1974). Recombinant caspase 3 (rC3) was expressed in *E.coli* and purified using the AKTA Explorer chromatography system (GE Healthcare). rC3 activity was determined using Promega's Caspase 3/7 activity assay. Aliquots of 5 mg of myofibrils in a mixed salt solution (MSS: 12mM NaCl, 12.6 mM $MgSO_4$•$7H_20$, 70 mM $KH_2PO_4$, 3.4 mM NaOH, 64.2 mM KOH, 11.1 mM $H_2SO_4$, 132 mM lactic acid, 100 mM 2-(N-Morpholino)ethanesulfonic acid (MES), pH5.8) were incubated with 10 units of rC3 at 4°C for 0, 1, 2, 5 and 8 d. Myofibrils were then centrifuged at 6 000 x g for 3 min at 4°C. The supernatant was removed and the pellet resuspended in 100 µl MSS+1.3 mM $CaCO_3$. The rC3 incubation reactions were not replicated. Proteins were separated by 12.5% SDS-PAGE and were visualised by Coomassie staining or transferred onto nitrocellulose by Western blotting. Blots were immunoprobed with anti-caspase 3 antibody (1:2000, Merck Biosciences, Nottingham, UK) or anti-rabbit desmin antibody (1:500, Sigma Aldrich, Poole, UK). Protein bands were detected using ECL Plus detection system (GE Healthcare) and intensities quantified (Quantity-One Multi Analyst, BioRad). Bands from Coomassie stained gels were analysed using MALDI-TOF Mass Spectrotrometer.

**Results** Incubation of myofibrils with rC3 over 8 d at 4°C resulted in increased protein degradation compared to myofibrils incubated with no rC3 as a negative control. Degradation products at 18 and 28 kDa were detectable from 0 d, with a degradation product at approximately 32 kDa detected from d 2 onwards. These bands were subsequently identified to result from degradation of myosin light chain, troponin T and actin, respectively, by MALDI-TOF peptide mapping. The band identified as troponin-I, by MALDI-TOF analysis, was found to decrease with incubation time and disappeared completely by 5 d (Fig.1). Western blot analysis showed that with time the inactive 32 kDa caspase 3 isoform decreased suggesting it is being activated into its active 20 kDa isoform. Protein levels of the myofibrillar protein desmin detected by Western blotting were found to decrease with increasing incubation time (Table 1).

| Time d | Caspase 3 32kDa | Desmin |
|---|---|---|
| 0 | 100 | 100 |
| 1 | 116.8 | 84.6 |
| 2 | 86.5 | 53.7 |
| 5 | 34.5 | 19.5 |
| 8 | 34.8 | 14.0 |

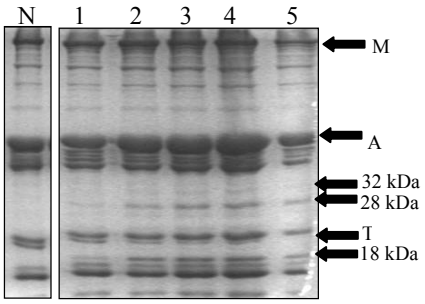


**Figure 1** SDS-PAGE of 20 µg of myofibrils incubated with rC3 at 4°C for 0, 1, 2, 5 & 8 d lanes 1-5 respectively. N-no rC3, M-myosin, A-actin, T-troponin I.

**Conclusion** This study has demonstrated evidence that rC3 is capable of causing degradation of myofibrillar proteins. Recombinant caspase 3 is active and causes proteolysis of myofibrils at postmortem conditions of 4°C and pH 5.8. Upon incubating for up to 8 d there was increased degradation of desmin and troponin I and also the appearance of a number of peptides that could result from proteolysis of actin, myosin and troponin T, as detected by Western blotting and Coomassie staining. These degradation patterns correspond with previous research identifying troponin I, T and desmin to be degraded in myofibrils incubated with µ-calpain. These findings therefore strengthen the hypothesis that postmortem proteolysis and meat tenderisation is a multienzymatic process and that caspases could contribute to it.

**Acknowledgements** CM Kemp was supported by BBSRC. rC3 plasmid was a kind gift from H.R Stennicke

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# Effect of selenium supplementation on the performance of ewes from hill sheep systems

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**Introduction** Selenium (Se) is an essential trace mineral that when deficient, has been associated with increased disease evidence in sheep. Selenium deficient areas are widespread in UK and Ireland affecting, soils, forages and consequently livestock. Se supplementation of ewes throughout pregnancy has been reported to reduce peri-natal lamb mortality and improve lamb growth rates (Muñoz *et al.,* 2005). The aim of this trial was to evaluate the effect of long term Se supplementation of adult ewes within a range of hill sheep systems.

**Material and methods** The study was carried out on 6 hill farms across Northern Ireland involving 1070 ewes. Six weeks prior to mating, sheep were balanced and randomly assigned to one of 2 treatments: selenium supplement (n=543) or control (n=529). The Se group received a subcutaneous injection (dose rate of 1 mL/50 kg of LW) of Deposel®, a long term inorganic source containing 50 mg/mL of barium selenate. The control group received no Se supplementation. Ewes on all farms were of 5 different genotypes as described by Speijers *et al.,* (2007). Blood samples for analysis of glutathione peroxidase (GPx) and Se were collected from a designated group of ewes (n=242) and their lambs at the start of the trial, at mating, at scanning, 6 weeks after lambing and at weaning. All ewes and lambs were weighed and body condition scored at each of the collection dates. The data were analysed using Genstat REML and binomial modelling, examining the effects of Se and ewe breed, while adjusting for farm, ram and age. Ewe blood data were adjusted for mean values from pre-mating sampling. For lamb LW gain (regression), litter size and sex were included as covariates.

**Results** Mean GPx (359.4, s.d. 249 U/g Hb) and Se (0.92, s.d. 0.5 µmol/L) values pre-mating (Figures 1) indicate that ewes had a low Se status, 48 and 68% below the reference range, respectively (Puls, 1988). Lambs born to Se ewes had higher Se and GPx levels than control lambs (Figures 1); however, Se means were still below the reference range (0.55, s.d. 0.3 µmol/L). Overall productivity in the supplemented group was better than in the control group when considered on ewe mated basis, due to a significant (P<0.05) increase in ewe fertility (Table 1). Although lamb birth weights were not affected by Se treatment, lambs born from Se ewes achieved higher growth rates (P<0.05) at 6 w than control lambs. Overall, growth rates to weaning were not affected. Supplementation had no effect on either ewe or lamb mortality, although abortion rates were significantly (P<0.001) reduced in supplemented ewes. Selenium treated ewes were more efficient in maintaining their body weight (P<0.01) and condition (P<0.05) than control ewes.

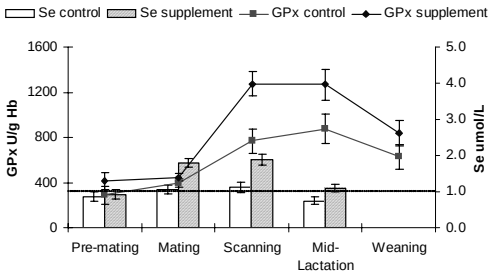


**Figure 1.** Effect of supplementation with Se on GPx and Se concentration in blood of ewes

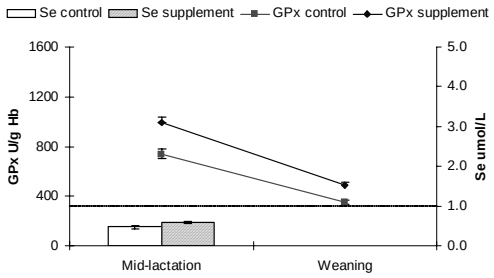


**Figure 2.** Effect of supplementation with Se in ewes on GPx and Se concentration in blood of lambs

**Table 1** Comparison of productivity between control ewes or ewes offered selenium supplementation

| | Control | Se supplement | S.E.D. | Significance |
|---|---|---|---|---|
| Conception rate | 0.88 | 0.92 | 0.035 (S.E.) | * |
| Nº lambs born per ewe lambed | 1.61 | 1.66 | 0.033 | |
| Nº lambs weaned per ewe mated | 1.14 | 1.25 | 0.046 | * |
| Lamb birth weight (kg) | 4.07 | 4.05 | 0.039 | |
| Lamb LW change to 6 w (g/d) | 272.8 | 280.9 | 3.16 | * |
| Ewe abortion rate | 0.04 | 0.01 | 0.107 (S.E.) | *** |
| Ewe LW change (pre-mating to weaning) (g/d) | 2.0 | 13.8 | 3.60 | ** |
| Ewe BCS change (pre-mating to weaning) | -0.018 | -0.012 | 0.0022 | * |

**Conclusions** In ewes with low Se status, supplementation with Se 6 w prior to mating positively affected the number of lambs born to ewes mated, and the growth rate of lambs in mid lactation. Se supplementation was also associated with reduced abortion rates and lower body weight and condition losses during the study.

**Acknowledgements** Programme Alβan, scholarship No.(E04D047780CL) and DARDNI.

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# The effects of iodine supplementation to the pregnant ewe on immunoglobulin G, vitamin E, T3 and T4 levels in the newborn lamb

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**Introduction** Lambs are born hypoimmunocompetent and are dependent on the absorption of immunoglobulin G (IgG) from colostrum for immunity in early life, similar to the situation with vitamin E (vit. E), which is also involved in maximising immunocompetence (Puls, 1994). High-level iodine supplementation during late gestation period of the ewe results in the production of progeny with an impaired ability to absorb colostral IgG and vit. E post partum (Boland *et al.,* 2006). Iodine is essential in the synthesis of the thyroid hormones, thyroxine (T4) and triiodothyronine (T3; McDonald *et al.,* 2002) that regulate the metabolic pattern of most cells and play a vital role in the process of cellular differentiation, growth and development in the foetus and neonate (Stanbury, 1996). The objectives of this experiment were to determine the effects of offering supplementary iodine for the final three weeks of pregnancy on serum vit. E, IgG, T3 and T4 concentrations at 1h, 24h and 72h *post partum.*

**Materials and methods** Thirty twin-bearing ewes were offered a basal diet of grass silage *ad libitum* supplemented with 800g/day of a 190g/kg crude protein concentrate from day 126 of gestation until lambing. The ewes were allocated to two dietary treatments as follows: C: no mineral supplement; I-3: 26.6 mg iodine from day 126 of pregnancy to parturition (~3 weeks). Iodine was delivered as calcium iodate added to the concentrate on a daily basis. Ten lambs (five sets of twin mates) were selected at birth from each treatment. A 10 ml blood sample was taken from all lambs at 1h, and from one of each set of twin mates at 24 h and 72 h *post partum.* Samples were analysed for IgG and vit. E concentrations as previously described (Boland *et al.,* 2006). Serum T4 and T3 concentrations were determined by solid phase time-resolved flouroimmunoassay using AutoDELFIA kits (Wallac Oy, Turku, Finland). The data were analysed using the General Linear Model procedure (PROC GLM) of the Statistical Analysis Systems Institute (1999-2001) (SAS, Version 8). The experiment was analysed as a randomised block design. All data were subjected to analysis of variance providing treatment means with standard errors of the mean (SEM).

**Results** Lamb serum IgG, vit. E, T3 and T4 concentrations at 1 h post partum, and lamb serum IgG and vit. E concentrations at 24 and 72 h post partum and IgG absorption efficiency are presented in Tables 1 and 2 respectively. At 1 h *post partum,* there were no significant differences in serum IgG, vitamin E, total or free T4 values ($P > 0.05$) between lambs born to C or I-3 ewes, while free T3 ($P < 0.05$) and total T3 ($P < 0.01$) were lower in the progeny of I-3 ewes compared to C ewes. At 24 h post partum serum IgG concentration tended ($P = 0.06$) to be higher in the C lambs and IgG absorption efficiency was significantly higher in the C vs I-3 lambs ($P < 0.05$). At 72 h post partum serum IgG concentrations were higher in the C vs I-3 lambs ($P < 0.01$).

**Table 1** The effect of treatment on lamb serum IGG, vitamin E, T3 and T4 concentrations at 1 h post partum (Least square means ± s.e.m.)

| | C | I-3 | s.e.m. | Sig. |
|---|---|---|---|---|
| 1 hr IgG (g/l) | 0.830 | 0.770 | 0.120 | ns |
| 1 hr Vit E (µg/ml) | 0.371 | 0.409 | 0.023 | ns |
| Total T4 (nmol/l) | 160.2 | 166.9 | 16.7 | ns |
| Free T4 (pmol/l) | 13.3 | 13.0 | 2.60 | ns |
| Total T3 (nmol/l) | $4.43^{a}$ | $2.36^{b}$ | 0.553 | * |
| Free T3 (pmol/l) | $28.8^{x}$ | $18.0^{y}$ | 2.36 | ** |

** = P<0.01; * = P< 0.05; ns = non significant

**Table 2** The effect of treatment on lamb serum IGG and vitamin E concentrations at 24 and 72 h post partum (least-square means ± s.e.m.)

| | C | I-3 | s.e.m. | Sig. |
|---|---|---|---|---|
| 24 h IgG (g/l) | 30.4 | 12.3 | 5.9 | 0.06 |
| 24 h vitamin E (g/l) | 2.27 | 2.21 | 0.21 | ns |
| 72 h IgG (g/l) | 25.2 | 8.31 | 3.51 | ** |
| 72 h vitamin E (g/l) | 3.42 | 2.88 | 0.325 | ns |
| 24h IgG absorption efficiency (%) | 26.2 | 9.27 | 4.13 | * |

**Conclusion** There are negligible quantities of IgG and vit. E in the serum of the lamb at birth. Offering supplementary iodine for three weeks prepartum reduces total and free T3 levels in the serum of the lamb at birth. This provides a potential link between high-level iodine supplementation in late pregnancy and reduced serum IgG levels in the newborn lamb. A lowered efficiency of IgG absorption during the first 24 h of life results in reduced serum IgG concentrations at 24 h and 72 h post partum.

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# Improving ovine peri-conception diets by feeding an algal source of omega-3 fatty acids

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**Introduction** The UK dairy industry currently suffers huge losses due to declining fertility. Although fertility in the sheep industry is good overall, the use of multiple ovulation and embryo transfer techniques would benefit from improved conception and embryo survival rates. Studies suggest that omega-3 supplementation may bring about a reduction in the secretion of prostaglandin F2α (PGF2α), which would favour the maintenance of the corpora lutea (CL) and hence, a successful pregnancy. Feeding fish meal to dairy cows has been found to improve pregnancy rates by 10% (Burke *et al.*, 1997). Using sheep as a model, this study aimed to evaluate whether reproductive performance in ruminants is improved by feeding an algal source of omega-3 fatty acids, particularly docosahexaenoic acid (DHA, C22:6 n-3). The study was also structured to determine whether prostaglandin production is affected by supplementing diets with omega-3.

**Materials and methods** Eighty-four English mule ewes were allocated to one of two treatment groups balanced for condition score and parity. The two treatment groups were: those receiving 6gDHA/ewe/day from an algal biomass source, and those receiving a control supplement (C) based on corn oil to balance the lipid content of the two diets. Ewes were condition scored every 2 weeks. Experimental diets were fed for 4 weeks prior to ram introduction until slaughter. Ewes were artificially synchronised and joined by rams for their second oestrus period. Blood samples were taken from a subset of ewes (n=58) to monitor plasma progesterone concentrations after mating. A further subset of ewes (n=16) were fitted with jugular cannulae 23 days after mating to monitor responses in plasma PGF2α metabolite (PGFM) concentrations in response to an oxytocin challenge (10 IU given intravenously). All ewes were slaughtered in a balanced design over a 3 week period, beginning 5 weeks after being mounted. Reproductive tracts were collected and dissected to monitor reproductive performance.

**Results** All ewes were mated. Pregnancy and ovulation rate (the number of CL per ewe) did not differ significantly between treatment groups. There was a tendency for ewes from the DHA treatment group to have a higher number of embryos than C ewes, however this was not significant (P = 0.069). There were no differences between treatment groups for embryo weight or size measurements. Ewes failing to conceive had lower ovulation rates, indicated by the number of CL at slaughter, compared to those that conceived (Non-pregnant: 1.9 CL/ewe vs Pregnant: 2.3 CL/ewe, P<0.05). Plasma progesterone concentrations after mating were similar between treatment groups for both non-pregnant and pregnant ewes. Plasma PGFM concentrations increased in response to the oxytocin challenge during early pregnancy in all ewes (P<0.001), however there were no significant differences between treatment groups in the concentrations of PGFM either before or after the oxytocin challenge.

**Table 1** Reproductive performance of ewes with or without a dietary supplement of algal biomass

| | *C* | *DHA* | *sem* | *P* |
|---|---|---|---|---|
| Pregnancy rate (%) | 78 | 88 | - | ns |
| Ovulation rate (equal to CL number) | 2.23 | 2.27 | 0.102 | ns |
| Embryo Number per pregnant ewe§ | 1.92 | 2.02 | 0.145 | ns |
| Embryo Number per ewe mounted§ | 1.50 | 1.88 | 0.191 | 0.069 |
| Cotyledons per embryo | 43.6 | 47.2 | 2.71 | ns |
| Cotyledons per embryo^ | 39.8 | 45.5 | 2.54 | 0.085 |
| Embryo Weight (g) # | 8.30 | 8.38 | 0.256 | ns |
| Embryo CRL (cm) | 4.81 | 4.89 | 0.151 | ns |
| Mean PGFM before oxytocin challenge (pg/ml) | 6.28 | 4.85 | 0.485 | 0.058 |
| Mean plasma PGFM after oxytocin challenge (pg/ml) | 51.3 | 41.0 | 12.0 | ns |

CRL = Crown Rump Length, § = with condition score at mating included as a factor (P<0.1)
^ = with littersize included as a factor (P<0.001)
# = with day of gestation at slaughter included as a covariate (P<0.001)

**Conclusion** Ewes fed an algal source of long chain omega-3 fatty acids prior to and after conception had a 10% higher pregnancy rate than ewes fed corn oil, although this difference was not statistically significant. There was no increase in ovulation rate, and contrary to the work of others (Mattos *et al.* 2003), data did not demonstrate any significant effect of omega-3 supplementation on PGF2α production. Therefore, the mechanism by which DHA is thought to improve aspects of fertility requires further investigation.

**Acknowledgements** We thank ABN Ltd for financial support, staff at Cockle Park for technical assistance and staff at the Royal Veterinary College London for assistance with PGFM analysis.

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# The effects of herbage allowance and frequency of allocation, and silage feed value when offered to mid gestation ewes on lamb birth weight and subsequent performance.

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**Introduction** It is essential to improve efficiency and reduce costs of production with decoupling of subsidy from production post Mid Term Review of the Common Agricultural Policy. Recent studies at this centre (Flanagan 2003, Keady *et al.* 2006) have shown that extended grazing (grazing during winter), either during mid, late or throughout pregnancy, provides a low cost system of wintering ewes. The aims of this study were to evaluate the effects of herbage allowance and frequency of allocation, and potential interactions during mid gestation on ewe performance, and lamb birth weight and subsequent performance. Furthermore a direct comparison of herbage allowance and grass silages of differing feed value was also undertaken.

**Materials and methods** Old permanent predominantly perennial ryegrass swards which were harvested for silage on September 6 received nitrogen (34 kg/ha) for extended grazing. The pasture was grazed *in situ* at dry matter (DM) allowances of 1.0 and 1.8 kg/head/day. The herbage was allocated daily or twice weekly. High (precision chopped) and low (big baled) feed value grass silages were ensiled on May 30 and August 5, from the primary growth and regrowth, respectively. The silages were offered *ad libitum* as the sole diet. The six treatments were offered to 120 ewes (initial liveweight 74.1 kg; condition score 3.7) from December 1 to February 1 in a fully randomised design. On February 1 all ewes were housed and offered the high feed value grass silage supplemented with concentrate at a total of 19 kg/head up to lambing. The ewes were synchronised to lamb on February 27. The data was analysed using general linear model procedures of SAS. *A priori* contrasts were used to test the effects of herbage allowance, frequency of movement and forage type. All ewes rearing single or twin lambs were grazed together until weaning without concentrate supplementation. Ewes rearing triplets were offered 1 kg concentrate daily whilst the lambs received a maximum of 300 g concentrate/head per day until weaning at 14 weeks.

**Results** The low and high feed value grass silages had DM and predicted metabolisable energy concentrations and intake values of 240 and 320 g/kg, 9.9 and 11.2 MJ/kg DM and 75 and 92 g/kg W $^{0.75}$, respectively. The swards had mean herbage DM mass and DM concentrations of 3373 kg/ha and 132 g/kg respectively. The effects of herbage allowance and frequency of allocation and silage feed value on animal performance are presented in Table 1. Increasing herbage allowance significantly increased ewe liveweight and condition score (CS) at the end of extended grazing and tended (P=0.06) to increase CS at lambing. Weight of lambs at birth and weaning and daily liveweight gain were significantly increased due to increased herbage allocation. Herbage allowance did not alter (P>0.05) ewe CS or weight at weaning or litter size. Frequency of herbage allocation or silage feed value did not alter ewe or lamb performance. There was a significant interaction between herbage allowance and frequency of allocation for ewe CS and weight at weaning, lambing assistance, and lamb birthweight. At the low allowance reducing frequency of allocation reduced ewe weaning CS and weight whilst at the high allowances reducing frequency of allocation increased ewe weaning CS and weight. Reduced frequency of allocation increased lambing assistance and lamb birth weight at the low herbage allowance but reduced lambing assistance and lamb birth weight at the high allowance. As determined by lamb weaning weight, the low and high feed value grass silages had the same feed value as 1.4 and 1.5 kg DM of extended grazed herbage allocated daily, respectively.

**Table 1** Effect of herbage allowance and frequency of allocation, and silage feed value in mid pregnancy on animal performance

| | Treatment | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Grass allowance (GA) (kg DM/d) | | | | Silage feed value (S) | | | Significance | | | | |
| Frequency of movement (F) | 1.0 Daily | 1.0 Twice weekly | 1.8 Daily | 1.8 Twice weekly | Low | High | s.e.m. | GA | F | S | GA x F | S V G |
| Ewe condition score at: | | | | | | | | | | | | |
| end of grazing | 3.08 | 2.90 | 3.38 | 3.32 | 3.19 | 3.25 | 0.086 | *** | NS | NS | NS | NS |
| lambing | 2.91 | 2.75 | 3.09 | 3.06 | 3.14 | 3.07 | 0.127 | P=0.06 | NS | NS | NS | NS |
| weaning | 3.33 | 2.98 | 2.97 | 3.10 | 3.12 | 3.10 | 0.109 | NS | NS | NS | * | NS |
| Ewe weight (kg) at: | | | | | | | | | | | | |
| end of grazing | 69.5 | 67.2 | 74.4 | 73.6 | 73.2 | 74.7 | 0.81 | *** | P=0.06 | NS | NS | NS |
| weaning | 76.8 | 72.3 | 73.5 | 75.1 | 77.2 | 75.2 | 1.43 | NS | NS | NS | * | NS |
| Litter size | 1.72 | 2.10 | 1.99 | 1.71 | 1.84 | 2.15 | 0.152 | NS | NS | NS | NS | NS |
| Assistance at lambing (%) | 11 | 40 | 19 | 6 | 11 | 12 | | NS | NS | NS | * | NS |
| Lamb weight (kg) at: birth | 4.26 | 4.68 | 5.12 | 4.73 | 4.52 | 4.50 | 0.138 | ** | NS | NS | ** | NS |
| weaning | 32.8 | 34.4 | 36.0 | 35.1 | 34.2 | 34.7 | 0.87 | * | NS | NS | NS | NS |
| Lamb LWG (g/d) | 287 | 301 | 313 | 308 | 312 | 315 | 7.7 | * | NS | NS | NS | NS |

**Conclusions** It is concluded that increasing herbage allowance during mid pregnancy increased lamb weaning weight and daily liveweight gain. The herbage allowance by frequency of allocation interaction for lamb birthweight maybe explained by the effects of the combination of low feed allowance and twice weekly herbage allocations on maternal partitioning of nutrients to foetal growth. Frequency of herbage allocation had no effect on performance. Offering the low and high feed value grass silage had the same feed value as 1.4 and 1.5 kg DM of extended grazed herbage allocated daily, respectively.

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# Performance of crossbred ewes in the hill sheep sector

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**Introduction** Flock genetics has a very major effect on the level and quality of output from the hill sheep sector. For example, ram breed substitution can increase output per ewe by up to 24% with concomitant improvements in carcass quality (Carson *et al*., 2001). Major opportunities now lie in improving the output potential of hill ewes through breeding. Currently, hill sheep flocks in the UK and Ireland consist mainly of purebred Scottish Blackface, Welsh Mountain, Swaledale and, to a lesser extent, Cheviot ewes. Using purebreds, rather than crossbred ewes, means that the potential benefits of heterosis are not exploited in harsh hill conditions where they are likely to be of greatest benefit. Crossbreeding can also be used to take advantage of breed differences in genetic merit for different traits e.g. hardiness, ease of lambing, prolificacy and carcass traits. In view of this background, a research programme was initiated to investigate the relative merits of a range of crossbred ewe types within hill sheep systems in Northern Ireland. This paper presents the results from the second phase of the study in which the performance of mature ewes (1-3 lambings) was investigated.

**Materials and methods** The study was carried out on six hill farms, located in the main hill regions of Northern Ireland. On each of the farms 200 purebred Blackface ewes were allocated to five mating groups over a period of 3 years (2001 – 2003). The mating groups were balanced for ewe live weight, condition score and age and allocated to Blackface, Swaledale, Cheviot, Lleyn or Texel sires. In the first year of the study, single sire mating groups were used separately on each farm, whilst in the second and third year a team of rams was used across all the farms using artificial insemination. A total of 15 Blackface, Swaledale, Cheviot, Lleyn and Texel rams were used to produce the crossbred progeny. Rams were selected, wherever possible, on the basis of their selection indices to represent the top 10% of recorded animals. The females produced from these matings were first mated at approximately 18-months of age. Over a 3-year period (October 2003-2005) these ewes were mated to a range of sire breeds, which were balanced across all the genotypes. Ewes were weighed and condition scored prior to mating and lambing, post-lambing and at weaning. Lambs from each of the crosses were weighed at birth, at six weeks of age and at weaning. The degree of intervention required at lambing was assessed on a five point scale where 1 = no assistance required and 5 = caesarian section required. The data were analysed using the GenStat REML (Residual Maximum Likelihood) procedure. This fitted fixed effects for farm, sire breed and crossbred ewe genotype.

**Results** The proportion of productive ewes was greater in Lleyn ($P<0.05$) and Texel X ($P<0.01$) compared with purebred Blackface ewes (Table 1). Litter size was highest with Lleyn X ewes ($P<0.001$), but Texel X and Swaledale X ewes also had higher litter size ($P<0.01$ and $P<0.05$, respectively) compared with purebred Blackface ewes. The proportion of ewes requiring assistance at lambing was higher with Texel X ewes compared with the other breed types ($P<0.05$). Overall, Texel X and Lleyn X ewes produced a greater litter number and weight at weaning ($P<0.05$ and $P<0.001$, respectively) compared with purebred Blackface ewes. Cheviot X ewes showed no increase in productivity over purebred Blackface ewes. Replacement rate, for ewes born in the first 2 years of the study, was higher for Texel X compared with Cheviot X ($P<0.01$), Lleyn X ($P<0.05$) and Swaledale X ($P<0.001$) ewes. Purebred Blackface ewes had a higher replacement rate compared with Swaledale X ewes ($P<0.05$), but similar to the other breed types.

**Table 1** The effect of ewe genotype on performance and lamb output

| | BXB† | SWXB | CHXB | LLXB | TXB | s.e.d. | P-value |
|---|---|---|---|---|---|---|---|
| No. of ewes | 387 | 355 | 377 | 429 | 480 | | |
| Pregnancy rate | $0.83^{a}$ | $0.86^{abc}$ | $0.85^{ab}$ | $0.88^{bc}$ | $0.90^{c}$ | 0.022 | 0.018 |
| No lambs born/ewe | $1.50^{a}$ | $1.60^{bc}$ | $1.53^{ab}$ | $1.71^{d}$ | $1.62^{c}$ | 0.039 | <0.001 |
| Lamb birth weight (kg) | $3.9^{b}$ | $3.9^{b}$ | $4.1^{c}$ | $3.8^{a}$ | $4.0^{b}$ | 0.05 | <0.001 |
| Proportion of lambs born without assistance | $0.78^{b}$ | $0.79^{b}$ | $0.74^{ab}$ | $0.77^{b}$ | $0.70^{a}$ | 0.030 | 0.009 |
| No lambs weaned/ewe | $1.22^{a}$ | $1.31^{abc}$ | $1.23^{ab}$ | $1.40^{c}$ | $1.32^{bc}$ | 0.046 | <0.001 |
| Weight of lambs weaned/ewe | $45.3^{a}$ | $46.0^{a}$ | $45.6^{a}$ | $49.7^{b}$ | $48.8^{b}$ | 1.17 | <0.001 |
| Ewe replacement rate‡ | $0.14^{bc}$ | $0.06^{a}$ | $0.08^{ab}$ | $0.11^{ab}$ | $0.18^{c}$ | 0.035 | 0.002 |

† BXB, Scottish Blackface X Blackface; SWXB, Swaledale X Blackface; CHXB, Cheviot X Blackface; LLXB, Lleyn X Blackface; TXB, Texel X Blackface.
‡ Replacement rate after the first breeding season for ewes born in the first 2 years of the study (2002-2003).

**Conclusions** Retaining crossbred females with Lleyn and Texel genes as replacement hill ewes can improve (up to 10%) lamb output at weaning compared to purebred Blackface ewes. Texel X Blackface ewes required more assistance at lambing and had a higher replacement rate after their first breeding season compared with the other crossbred ewe types, although replacement rate was not significantly different to purebred Blackface ewes.

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## Relationships between mobilisation of body reserves in hill ewes and lamb production to weaning

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**Introduction** Previous studies using X-ray computed tomography (CT) of Scottish Blackface hill ewes have shown that carcass fat, internal fat and muscle are depleted during pregnancy and early lactation and deposited during late lactation and the dry period (Lambe et al., 2003a). Muscle weights and proportions throughout the year are positively genetically correlated with total litter weight reared, largely due to increased litter size. Average weights of lambs reared were positively correlated with carcass fat weight or proportion pre-lambing and internal fat weight pre-mating, but negatively correlated with internal fat proportion pre-lambing (Lambe et al., 2005). However, is genetic potential to mobilise and regain increased amounts of tissue during the year positively associated with the ability to produce more and heavier lambs? The aim of this study was to address this question by relating total loss and gain of fat and muscle in ewes during the annual cycle with lamb production traits.

**Materials and methods** Scottish Blackface ewes (n=513) were CT scanned four times per year: pre-mating (PM), pre-lambing (PL), mid-lactation (ML), weaning (WE), from 18 months to 5 years of age (maximum 16 scans per ewe). Prediction equations (Lambe et al., 2003b) estimated total weights of carcass fat (CF), internal fat (IF) and muscle (M) from cross-sectional CT images, for each animal at each scanning event. Total weight lost (LOSS) from each tissue from PM to ML, and total weight gain (GAIN) from ML to PM were calculated. Bivariate analyses in ASREML (Gilmour et al., 2001) estimated genetic and phenotypic correlations between LOSS (in the same year as lambs produced) or GAIN (the previous year) of each tissue and lamb production traits - number of lambs weaned (NLW) and average lamb weaning weight (WWT). Animal models were fitted with fixed effects of age, year and grazing location, with additional fixed effects for LOSS and GAIN (number weaned the previous year and interaction with age) and WWT (interaction of NLW with age). LOSS and GAIN of total soft tissue weight (ST = CF + IF + M) was also tested in the models for LOSS and GAIN, to estimate changes in each tissue relative to total change (results shown as LOSS r and GAIN r), and correlations with lamb production traits estimated.

**Results** Moderate positive phenotypic correlations were estimated between NLW and LOSS of each tissue (CF, M, IF) or ST (Table 1). However, NLW was not significantly correlated genetically with LOSS from any tissue. LOSS of CF had a higher genetic correlation with NLW than the other tissues, and relative to LOSS of ST (LOSS r) was moderately correlated with NLW, but due to large standard errors these estimates were not significant. Phenotypic correlations between LOSS of M or IF and WWT were low, but LOSS of CF had a moderate positive correlation with WWT. Moderate genetic correlations were estimated between WWT and LOSS in each tissue, as well as in ST. When adjusted for LOSS of ST, none of the three tissues were correlated with WWT, suggesting that none of the three tissues is significantly more important in the relationship between tissue loss and WWT. Phenotypic correlations between lamb production traits and GAIN in tissue weights the previous year were all close to zero. Genetic correlations with GAIN of CF or M were moderate and positive for NLW and higher for WWT. Including GAIN in ST in the model (GAIN r) suggested that GAIN in CF was most important for NLW the following year and that a larger proportion of IF in total GAIN in ST was associated with lower NLW and WWT. Large standard errors limit interpretation of some of the genetic correlations.

**Table 1** Genetic and phenotypic correlations (and standard errors) between LOSS or GAIN and lamb production traits. *Standard errors for phenotypic correlations were all 0.02, so are not shown in the table

| | | GENETIC | | | | PHENOTYPIC* | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | ST | CF | M | IF | ST | CF | M | IF |
| NLW | LOSS | 0.10(0.27) | 0.19 (0.25) | -0.05(0.25) | 0.02 (0.28) | 0.32 | 0.33 | 0.19 | 0.27 |
| | LOSS r | | 0.23 (0.25) | -0.14(0.21) | -0.06(0.27) | | 0.11 | -0.10 | -0.001 |
| | GAIN | 0.09(0.25) | 0.23 (0.25) | 0.15 (0.26) | -0.20(0.28) | 0.07 | 0.06 | 0.05 | 0.05 |
| | GAIN r | | 0.34 (0.28) | 0.09 (0.23) | -0.71(0.49) | | -0.002 | 0.01 | -0.01 |
| WWT | LOSS | 0.52(0.19) | 0.49 (0.19) | 0.42 (0.19) | 0.40 (0.21) | 0.17 | 0.21 | 0.09 | 0.09 |
| | LOSS r | | 0.01 (0.21) | 0.07 (0.17) | -0.09(0.22) | | 0.10 | -0.04 | -0.06 |
| | GAIN | 0.48(0.19) | 0.46 (0.19) | 0.48 (0.19) | 0.17 (0.22) | 0.08 | 0.06 | 0.08 | 0.05 |
| | GAIN r | | 0.15 (0.23) | 0.17 (0.18) | -0.61(0.39) | | -0.02 | 0.04 | -0.02 |

**Conclusions** Scottish Blackface ewes with a genetic propensity to mobilise soft tissue (CF, M and IF) during pregnancy and lactation also have a genetic propensity to rear heavier lambs. Ewes that regain more muscle and carcass fat from ML to PM are also inclined to rear more and heavier lambs the following year.

**Acknowledgements** Thanks to Defra for funding and staff at the CT unit at SAC for data collation.

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# Effects of maternal undernutrition on lamb carcass characteristics and muscle fibre composition at 17 and 24 weeks of age

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**Introduction** We have previously shown that the majority of muscle differentiation and fibre formation in sheep takes place around d 85 of gestation, with myoblast proliferation mainly occurring before this time (Fahey et al., 2005a). In a second study (Fahey et al., 2005b), maternal nutrient restriction during the proliferation stage immediately before the period of major fibre formation (d30-70 gestation) resulted in a reduction in the numbers of fast fibres in 14 d lambs. Maternal undernutrition during (d55-95) and after (d85-115) major fibre formation did not alter the muscle fibre characteristics of 14 d lambs. The objective of the present study was to determine whether the changes in muscle fibre development seen previously in 14 d lambs from ewes undernourished from d 30 to 70 of gestation are still evident in older animals, and what the long term consequences are in relation to growth rates and carcass composition.

**Materials and Methods** Two trials were conducted on twin-bearing ewes and in both trials the control ewes were fed on an individual basis throughout pregnancy to the recommended dietary allowance relative to metabolic body weight. The treatment groups were fed as for controls except that their nutrient supply was reduced to 50% of the recommended allowance from d 30 to 70 (Exp. 1) or d 30 to 85 (Exp. 2) of gestation, after which they were returned to the same level of nutrition as the control group. After weaning, twin lambs were individually housed and fed ad libitum to 24 or 17 wk of age for Exp 1 and 2 respectively. Liveweight and food intake were measured and ultrasound scanning was used to monitor muscle and back fat depth. At slaughter, muscle samples (*Longissimus dorsi, Semitendinosus, and Vastus lateralis*) were rapidly removed from the left side of the carcass, snap-frozen in liquid nitrogen-cooled isopentane and stored at –80$^{o}$C for determination of muscle fibre type characteristics. Samples of subcutaneous (tail fat), perirenal and omental adipose tissue were also rapidly removed from the left side of the carcass, frozen quickly in liquid nitrogen, and stored at –80$^{o}$C for determination of adipocyte size. Whole weights of the three muscles, perirenal and omental adipose tissue, and various other organs were also recorded. The width (maximum distance from end adjacent to spinal process, outwards along the rib), and depth (longest distance, perpendicular, on same surface) of *Longissimus dorsi*, and thickness of back fat over its deepest part were recorded. Dry matter, fat (Soxhlet) and protein (Kjeldahl) contents of the intact right side of the carcass and samples from the three dissected muscles were also determined. Data were analysed by linear mixed model using the REML tool in Genstat (Release 8.1, Lawes Agricultural Trust).

**Results** Allowing the lambs to grow to 24 wk (Exp. 1) demonstrated some significant effects of maternal undernutrition on carcass characteristics (most significant data shown in Table 1), which were not apparent ($P > 0.129$) when the animals were slaughtered at 17 wk (Exp. 2, data not shown). In contrast to previous data in 14 d lambs, maternal undernutrition had no major effects upon the fibre type composition of the muscles at either age.

**Table 1** Significant effects of maternal restriction d 30-70 gestation on lambs grown to 24 wk age

| | Treatment | | | | P-value | | |
|---|---|---|---|---|---|---|---|
| | Female | | Male | | | | |
| | Control | Restricted | Control | Restricted | Treatment | Sex | Tr x S |
| Birth wt, kg | 4.8 | 4.6 | 5.2 | 5.3 | ns | *** | ns |
| Slaughter wt, kg | 50.8 | 47.1 | 64.1 | 63.4 | * | *** | ns |
| Growth, kg/d | 0.30 | 0.29 | 0.41 | 0.38 | ** | *** | ns |
| Intake, kg/d | 1.6 | 1.5 | 1.9 | 1.9 | ns | *** | ns |
| *Longissimus dorsi* wt, g | 664 | 644 | 848 | 777 | * | *** | ns |
| *Semitendinosus* % fat | 7.1 | 7.6 | 6.7 | 8.0 | * | ns | ns |
| *Vastus lateralis* wt, g | 192 | 179 | 238 | 226 | ** | *** | ns |
| Perirenal adipocyte diameter | 119 | 128 | 106 | 118 | * | * | ns |

**Conclusions** There were no effects of pre-natal dietary restriction prior to major fibre formation on the subsequent carcass quality of the adult lambs when slaughtered at commercial weights (17 wk age). This suggests the animals have somehow compensated for the changes in muscle fibre composition previously seen at 14 d. However, the lambs in this study were well-fed during post-natal growth. Whether the offspring would still have been able to compensate if they had received poor nutrition post-natally and whether that failure to compensate would have influenced the carcass composition are questions that remain to be answered. Evidence of an alteration in fat:lean ratio was seen when the lambs were grown for longer (to 24 wk).

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# The effects of offering a concentrate or grass silage based diet to single, twin and triplet-bearing ewes in late pregnancy on ewe performance

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**Introduction** The nutrient requirements of ewes increase greatly during the final two months of gestation as a result of the rapid foetal growth (Robinson, 1990). Traditionally these nutritional requirements were met by feeding grass silage supplemented with concentrates (Sheehan *et al*., 1979). Intakes of silage by sheep are variable, dependant on a range of factors with silage digestibility of major importance (Sheehan, 1975). However, voluntary silage intakes have been reported to vary irrespective of quality (Sheehan and Fitzgerald, 1977). Alternatively, the energy requirements of the ewe during late pregnancy can be supplied by offering a complete concentrate diet (Sheehan, 1975). The objectives of this experiment were to compare the performance of single, twin and triplet-bearing ewes when offered either a concentrate diet or a silage based diet supplemented with concentrates, each offered at 90% of metabolizable energy requirements for maintenance and conceptus growth.

**Materials and methods** A 3 x 2 factorial experiment was designed to compare the performance of single, twin and triplet-bearing ewes when offered a concentrate based diet with or without ewes having access to grass silage as a source of long fibre. Fifty single (S), 65 twin (TW) and 63 triplet (TR) bearing ewes were allocated to one of two dietary treatments, either a complete concentrate diet at 90% of their metabolizable energy requirements for maintenance and foetal growth (C) or grass silage offered *ad libitum* supplemented with concentrates to bring the total daily diet to 90% of ME requirements for maintenance and foetal growth (GS). The metabolizable energy (ME) content (MJ/kg DM) was calculated as 0.16 x DOMD value. Ewes were group penned using straw bedding with 2 pens per treatment. The concentrate offered to all ewes from days 107 to 132 of pregnancy contained 137g/kg crude protein (CP) and this was replaced with a 164g/kg CP concentrate from day 133 until parturition. Group silage intakes were recorded weekly. Daily rumination records were kept from day 121 to 139 of pregnancy. Records were taken of the number of ewes observed to be chewing their cud over a five-minute observation period at 08.00 h and 12.00 h each day. Ewes were milked at 1 h, 10 h and 18 h *post partum* and colostrum volumes recorded. The data were analysed as a 3 x 2 factorial using the General Linear Model procedure (PROC GLM) of the Statistical Analysis Systems Institute (1999-2001) (SAS, V8).

**Results** The influence of dietary treatment on feed intake, gestation length and colostrum production are shown in Table 1. Ewes on the grass silage based treatments had higher intakes of dry matter (DMI; P<0.001), crude protein (CPI) and a lower colostrum yield (P<0.01) and a longer gestation length (P<0.05) than ewes offered concentrate based diets. DM, CP and ME intakes increased (P<0.001) as litter size increased. Both triplet and single bearing ewes had lower total colostrum yields to 18 h post partum than twin bearing ewes (P<0.05). Regardless of dietary treatment single bearing ewes had a longer gestation length than twin bearing ewes (P<0.05). There was a significant interaction between diet and litter size for all parameters measured (P<0.05). The percentage of ewes observed ruminating was higher on GS diets (P<0.001).

**Table 1** The effect of dietary treatment on ewe intake, gestation length and colostrum yield (LSM ± s.e.m.)

| Diet (D) | C | | | GS | | | | Significance | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Litter size (LS) | S | TW | TR | S | TW | TR | s.e.m. | D | LS | D*LS |
| DMI (kg/hd/d) | 1.11 | 1.30 | 1.43 | 1.27 | 1.45 | 1.61 | 0.277 | *** | *** | *** |
| MEI (MJ/hd/d) | 13.8 | 16.8 | 19.0 | 13.8 | 16.9 | 17.8 | 0.35 | ns | *** | *** |
| CPI (g/hd/d) | 195 | 236 | 267 | 231 | 273 | 285 | 6.4 | ** | *** | *** |
| Gestation length (d) | 147.9 | 146.8 | 146.8 | 148.0 | 147.4 | 147.8 | 0.34 | * | * | * |
| Colostrum yield (ml) | 1960 | 2393 | 1830 | 1689 | 1843 | 1720 | 139.4 | ** | * | *** |
| Ewes ruminating | 0.38 | 0.30 | 0.25 | 0.57 | 0.72 | 0.71 | 0.03 | *** | NS | *** |

ns=non significant; * P<0.05; **P<0.01; ***P<0.001

**Conclusions** These data show that all concentrate diets can be successfully offered to single, twin and triplet bearing ewes in the final stages of gestation in the absence of a source of long fibre in the diet, without experiencing any adverse effects in terms of metabolic disease or reduced performance. However, the lower incidence of rumination with the concentrate treatments may be a cause for concern. The increased colostrum yields with the concentrate fed ewes may offer a benefit to the newborn lamb, as an adequate supply of quality colostrum is essential to the newborn to ensure survival. With the increasing cost of silage production and the demand for low labour production systems, concentrate feeding may offer a realistic alternative to grass silage-based diets for housed pregnant ewes.

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# The effects of offering a concentrate or grass silage based diet to twin-bearing ewes in late pregnancy on ewe and lamb performance

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**Introduction** Approximately 75% of ewes are housed in Ireland for some portion of the winter, normally the late gestation period. This coincides with a large increase in dietary requirements, with 80% of lamb birth weight laid down in the final two months of gestation (Robinson, 1990) and also udder development during this period. Traditionally, these nutritional requirements were met by feeding grass silage *ad libitum* and supplementing with concentrates on a stepped rate with advancing pregnancy. With the introduction of the decoupled single farm payment and an ever-decreasing labour supply, sheep producers are looking for financially attractive, low labour alternatives and a concentrate based diet may be one such alternative. The objectives of this experiment were to compare an all concentrate diet supplemented with different fibre sources with the standard silage based diet supplemented with concentrates on feed intake, ewe performance and lamb growth to weaning.

**Materials and methods** Sixty-four twin-bearing ewes were individually housed in late pregnancy and allocated to four dietary treatments (n=16). The ewes were housed in individual pens having timber-slatted floors and offered one of the following diets; T: silage *ad libitum* with supplementary concentrate (offered to meet 100% of ME requirements for maintenance and foetal growth); C90-St concentrate offered at 90% of ME requirements for maintenance and foetal growth with 150g of chopped oaten straw; C100-St concentrate offered at 100% of ME requirements for maintenance and foetal growth with 150g of chopped oaten straw; C100-GS concentrate offered at 100% of ME requirements for maintenance and foetal growth with 400g silage (equivalent to same DM as 150g chopped straw). Diets were offered from day 105 of gestation. The concentrates offered to all ewes contained 137g/kg crude protein (CP) from days 105 to 132 of pregnancy, and this was replaced with a 164 g/kg CP concentrate from day 133 of gestation until parturition. Intakes were recorded on a daily basis and lamb birth weight, colostrum production and quality and lamb growth rate to weaning (13 weeks) were recorded. Records were taken of the number of ewes observed to be chewing their cud over a five-minute observation period at 08.00 h and 12.00 h each day. IgG absorption was calculated using the equation IgG absorption = ((birth weight x 0·075 x serum IgG concentration)/total IgG fed) x 100. The data were analysed using the General Linear Model procedure (PROC GLM) of the Statistical Analysis Systems Institute (1999-2001) (SAS, V8).

**Results** Table 1 shows dietary intake, gestation length, and colostrum yield. C90-St and C100-GS treatments had lower DM intakes than T and C100-St treatments ($P<0.001$). C90-St ewes had a lower ME intake than other treatments ($P<0.001$). T ewes had a higher CP intake than all other treatments and C90-St had a lower CP intake than all other treatments ($P<0.001$). Treatment had no effect on gestation length ($P>0.05$). C100-St had a higher colostrum yield to 18 h than T or C90-St ewes ($P<0.05$). The C100-St ewes had a lower colostral CP concentration at 1 h than T or C100-GS ewes ($P<0.05$). The percentage of ewes observed ruminating was higher on the T diet ($P<0.001$) compared to the concentrate diets, whilst fibre source had no significant effect on rumination ($P>0.05$). Treatment had no effect on lamb birth weight or IgG absorption ($P>0.05$; Table 2). The C100-GS progeny had a lower growth rate and lower weaning weight than the T and C100-St progeny ($P<0.05$).

**Table 1** The effect of treatment on ewe performance (LSM ± s.e.m.)

| | T | C90-St | C100-St | C100-GS | s.e.m. |
|---|---|---|---|---|---|
| DMI (kg/hd/d) | $1.55^{b}$ | $1.41^{a}$ | $1.57^{a}$ | $1.46^{b}$ | 0.027 |
| MEI (MJ/hd/d) | $16.8^{b}$ | $15.7^{a}$ | $17.6^{b}$ | $16.9^{b}$ | 0.27 |
| CPI (g/hd/d) | $272^{c}$ | $197^{a}$ | $222^{b}$ | $221^{b}$ | 3.4 |
| Gestation length (d) | 147.7 | 146.8 | 146.8 | 146.5 | 0.51 |
| Colostrum yield (ml) | $2196^{x}$ | $2128^{x}$ | $2698^{y}$ | $2349^{xy}$ | 171.7 |
| 1 h CP concentration (g/kg) | $205^{y}$ | $190^{xy}$ | $169^{y}$ | $216^{x}$ | 10.4 |
| % Ewes Ruminating | $42.0^{a}$ | $14.1^{b}$ | $13.1^{b}$ | $12.8^{b}$ | 1.64 |

**Table 2** The effect of treatment on lamb birth weight, IgG absorption and growth rate to weaning (LSM± s.e.m.)

| | T | C90-St | C100-St | C100-GS | s.e.m. |
|---|---|---|---|---|---|
| Birth weight (kg) | 5.3 | 5.2 | 5.2 | 5.2 | 0.18 |
| IgG absorption efficiency (%) | 24.5 | 25.7 | 23.0 | 22.0 | 1.50 |
| Average daily gain to weaning (g/hd/d) | $297^{y}$ | $288^{y}$ | $279^{xy}$ | $263^{x}$ | 9.3 |
| Weaning weight (kg) | $32.5^{y}$ | $31.8^{y}$ | $30.4^{xy}$ | $29.5^{x}$ | 0.86 |

$^{x, y}$ $P<0.05$; $^{a, b, c}$ $P<0.001$

**Conclusions** This experiment shows that diets largely consisting of concentrates can be successfully offered to housed ewes during late pregnancy with ewe performance matching or exceeding that for ewes fed a diet of silage *ad libitum* supplemented with concentrates, In the current study the source of dietary fibre (straw/grass silage) used in the diet had no affect on ewe performance. The results of this study indicate that a concentrate-based diet may offer an alternative to traditional forage based diets for ewes in late pregnancy.

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# The effects of forage maize and wheat and triticale whole crop silages on the performance of lactating dairy cows

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**Introduction** Patterson *et al*. (2004, 2005) obtained positive intake and milk production responses to the inclusion of maize silage in grass silage-based diets under Northern Ireland conditions. However, fermented whole crop and high dry matter (DM) milled urea/urease-treated whole crop wheats both increased forage intake, but had no significant effect on milk production. The aim of the present study was to further investigate the milk production potential of milled high DM whole crop wheat, in both urea-treated and 'fermented' forms, and fermented triticale whole crop, as a partial replacement for grass silage.

**Material and methods** Silages were prepared from first cut grass, forage maize which had been sown under complete cover plastic mulch and winter wheat and triticale crops. The feeding study (7 treatment randomised block) was based on grass silage (GS) offered as the sole forage with either 7 or 10 kg concentrates/d, or as a 50:50 DM mixture of grass silage with forage maize silage (MS), fermented inoculant treated precision chopped whole crop wheat silage (FW), high DM urea/urease-treated milled whole crop wheat (UW), high DM inoculant-treated milled whole crop wheat (FHW) or fermented inoculant treated precision chopped triticale whole crop (TS). The wheat and triticale crops were harvested to produce a residual stubble height of 15-20 cm. The forages were offered *ad libitum* and forage mixtures were supplemented with 7 kg concentrates/d. The seven dietary treatments were offered to 21 lactating dairy cattle in a partially balanced changeover design consisting of 3 periods each of 4 weeks duration, with the final 2 weeks of each period being used as the recording period. Concentrates were fed in-parlour and the forages were mixed in a diet mixer and individual intakes were recorded. The results were subjected to statistical analysis using the REML technique in Genstat 5.

**Results** The grass silage had DM and ME (*in vivo*) of 225 g/kg and 11.0 MJ/kg DM. The MS, FW, UW, FHW and TS silages had mean DM; 340, 440, 776, 771 and 500 respectively, and starch; 291, 339, 368, 392 and 294 respectively. The effects of forage treatment and level of concentrates on animal performance are presented in Table 1. The alternative forage treatments significantly increased total forage DM intake (DMI) by a mean across treatments of 0.21 proportionally, but only MS produced a significant increase in milk yield and yield of fat plus protein. The butterfat content of milk was increased by all of the alternative forages except UW, while protein content was increased by all of the alternative forages. On the basis of responses in yield of fat plus protein, the concentrate sparing effects of the MS, FW, UW, FHW and TS silages were; 4.7, 3.0, 1.7, 0.2 and 2.0 kg concentrates/cow/d.

**Table 1** The effects of forage treatment and concentrate level on performance

| | GS Low conc. | GS High conc. | MS | FW | UW | FHW | TS | SED | Sig |
|---|---|---|---|---|---|---|---|---|---|
| Forage DMI (kg/d) | $10.0^{a}$ | $9.8^{a}$ | $12.1^{b}$ | $12.9^{c}$ | $12.1^{b}$ | $12.0^{b}$ | $11.9^{b}$ | 0.38 | *** |
| Total DMI (kg/d) | $16.2^{a}$ | $18.4^{bc}$ | $18.2^{bc}$ | $18.9^{c}$ | $18.1^{bc}$ | $18.0^{bc}$ | $17.8^{b}$ | 0.41 | *** |
| Milk yield (kg/d) | $27.6^{ab}$ | $28.7^{bc}$ | $29.0^{c}$ | $28.3^{bc}$ | $28.2^{abc}$ | $27.1^{a}$ | $27.9^{ab}$ | 0.58 | * |
| Fat (g/kg) | $37.7^{ab}$ | $38.8^{b}$ | $39.0^{b}$ | $38.3^{b}$ | $36.4^{a}$ | $38.4^{b}$ | $38.1^{b}$ | 0.81 | * |
| Protein (g/kg) | $31.3^{a}$ | $32.4^{b}$ | $32.5^{bcd}$ | $33.2^{bc}$ | $33.3^{cd}$ | $32.9^{bc}$ | $32.3^{b}$ | 0.41 | *** |
| Fat + protein (g/d) | $1898^{a}$ | $2039^{b}$ | $2063^{b}$ | $2004^{ab}$ | $1957^{ab}$ | $1905^{a}$ | $1968^{ab}$ | 56.3 | * |
| Forage efficiency† (MJ/kg) | $8.33^{b}$ | $9.16^{c}$ | $7.30^{a}$ | $6.78^{a}$ | $7.08^{a}$ | $6.90^{a}$ | $7.26^{a}$ | 0.383 | *** |

† Milk energy output/total forage DM intake (MJ/kg)
Significance: P<0.05,*; P<0.01,**; P<0.001,***
Means not having the same superscript letter are significantly different

**Conclusion** While the alternative forages increased total forage intake, only maize silage significantly increased the yields of either milk or fat plus protein. All of the alternative forages had positive effects on milk composition except urea-treated wheat for butterfat. The fermented triticale treatment gave similar performance to fermented whole crop wheat while forage maize produced the greatest concentrate sparing effect.

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# An evaluation of finishing strategies based on forage and concentrate for cull dairy cows

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**Introduction** Replacement rate on farms in Southern Ireland has increased from 16 per cent in 1990 to 27 per cent in 2003 or an increase of 0.8 per cent per year (Evans et al., 2004). Cull cow compromise about 38 per cent of all cattle slaughtered at Irish meat factories. Only 23% of total cows slaughtered in 2005 were killed in the first third of the year, 36% in the middle third and 41% killed in the final third, this indicates that there is an influx of unconditioned dairy cows from the milking herd at the end of the cow's lactation. In Ireland, the average for all cull cow carcass weights is 282kg. This is considerably less than the average for other EU countries. It is especially low when compared to values in major cull cow markets such as France (343kg). The objective of this study was to evaluate four different finishing treatments for cull dairy cows based on grass silage and concentrate.

**Materials and methods** The experiment was undertaken in Teagasc Moorepark Research Centre, Fermoy, Co. Cork; Ireland. The experiment commenced in December 2005 and was completed in June 2006. Sixty eight multiparous Holstein-Fresian cull spring calving dairy cows were randomised and assigned to a four treatment (n = 17) finishing experiment penned in groups of 17. Mean lactation number was 3.3 (s.d. 1.93), pre experimental live weight 597 kg (s.d. 68.9) and pre-experimental body condition score 2.7 (s.d 28.30), respectively. The four treatments were, ad-lib grass silage (GS); GS + 3 kg concentrate (GS+3C); GS + 6 kg concentrate (GS+6C); GS + 9 kg concentrate (GS+9C).Concentrate composition, on a fresh weight basis, was: 0.33 barley (rolled), 0.32 corn gluten, 0.32 citrus pulp and 0.03 dry cow minerals. Silage composition was 29.6 (DM) (g $kg^{-1}$), pH 3.9 and UFL 0.8 (g/kg). Liveweight, body condition score, back fat, skeletal, carcass classification and group / individual DM intake were measured**.** Finishing targets were set to ensure that the cows reached the carcass classification required by the abattoir to optimise carcass value; carcass weight > 272 kgs, fat score 3 or 4L and carcass classification P+ or O**.** Post slaughter measurements recorded were kidney channel fat, carcass classification and boning carcass data. 10 sub samples from each treatment under went meat quality analysis; proximate, tenderness, fat and muscle colour. Data was analysed using analysis of variance in SAS.

**Results**: As the slaughter criteria was pre determined, there was no significant treatment effect on liveweight and BCS. The two variables which had statistical significance were average daily gain (ADG) and days on trial for each treatment. As the proportion of concentrate in the diet increased there was a linear decrease in days to slaughter. There was a linear response to concentrates up to 6 kg concentrate day-1. Mean kill out for each treatment was 46 percent. With the finishing criteria set in the study the total feed budget for each treatment was in excess of 1.4 tonnes DM. As concentrate level increased there was a linear (P 0.001) increase in DM intake (DMI) between treatments. There was no significant effect of treatment on meat quality analysis.

**Table 1** Effect of feeding treatment on mean values for physical and carcass characteristics

| | Treatment | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | GS | GS+3C | GS+6C | GS+9C | Sed | Sig | Lin | Quad |
| Slaughter Liveweight (kg) | 699 | 703 | 708 | 698 | 10.9 | NS | NS | NS |
| Slaughter BCS | 3.5 | 3.5 | 3.5 | 3.5 | 4.49 | NS | NS | NS |
| Weight gain (kg/day) | $0.75^{a}$ | $0.91^{a}$ | $1.14^{b}$ | $1.15^{b}$ | 0.094 | *** | *** | NS |
| Period on trial (days) | $122^{a}$ | $108^{ac}$ | $95^{bc}$ | $84^{b}$ | 7.49 | *** | *** | NS |
| Carcass cold weight (kg) | 315 | 325 | 322 | 323 | 8.7 | NS | NS | NS |
| Total DMI (kg DM/day/cow) | $12.7^{a}$ | $13.7^{b}$ | $15.4^{c}$ | $17.6^{d}$ | 0.46 | *** | *** | NS |

**Conclusions** Feeding system significantly affected the finishing time period at which cull cows are finished. No advantages were found in ADG to feeding greater than 6kgs concentrate/cow/day. The finishing criteria must be reached to achieve optimum selling price. Cows targeted for this enterprise must be able to achieve these targets. The feed budget required to achieve finishing criteria regardless of treatment was 1.4 tonnes/cow.

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# Holo-analysis of the efficacy of exogenous dietary enzymes in beef and dairy cattle

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**Introduction** Holo-analytical models are used for the prediction of nutritional responses, product comparisons and the setting of future research priorities. Following holo-analyses of the efficacy of exogenous enzymes in monogastrics (Rosen, 2006), this study in ruminants comprises holo-analyses of all available data from beef and dairy cattle, negatively-controlled, start-to-finish tests in meat and milk production. The beef enzymes mainly contain amylases, cellulases and xylanases, with a total of eight other side activities and the dairy, betaglucanases, cellulases and xylanases, with 12 declared side activities.

**Materials and methods** The data banks furnished by a world-wide Rumzyme literature collection of 327 publications (1958-2003) have been derived from 11 beef and 27 dairy papers, containing 48 and 75 performance tests with totals of 966 and 1,348 cattle respectively. Holo-analysis elaborates holistic empirical multiple regression models with dependent variable responses quantified in terms of all available genetic, environmental, managemental, dietary and nutrient independent variables, deploying standard multiple regression methodology (JMP, 2000) for best-fit models with maximum multiple regression coefficient squares ($R^2$) and minimum root mean square errors (RMSE). These Brumzyme and Drumzyme holo-analyses utilized both conventional $P \leq 0.05$ in/$P \geq 0.10$ out and less stringent $P \leq 0.25$ in/$P \geq 0.33$ out partial regression coefficient significances. The latter is a useful tool to indicate potentially important variables in smaller (<100) test data banks. The beef cattle models on dry matter intake (DMI), liveweight gain (LWG) and dry matter feed conversion ratio (DMC) effects were elaborated using 17 independent variables. The dairy cattle models for effects on DMI, daily milk production (MKD), milk/feed conversion ratio (MKC), gravimetric 4% fat-corrected milk (GFM), milk protein (MPP), milk fat (MFP) and milk lactose (MLP) contents were developed using 30 independent variables. Enzyme dosage terms tested were logarithmic and quadratic.

**Results Table 1** Conventional and less stringent models for the effects of exogenous enzymes on (a) beef dry matter intake conversion (DMCeff) and (b) dairy milk yield/dry matter conversion (MKCeff)

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| a) | DMCeff | = | 1.34 | − 0.117PRP | − 0.167SIB | | | | | |
| | $R^2$ 0.477 | se | 0.525 | 0.036 | 0.092 | | | | | |
| | RMSE 0.451 | P | 0.016 | 0.003 | 0.079 | | | | | |
| | n 36 | | | | | | | | | |
| | DMCeff | = | 1.51 | − 0.131PRP | − 0.248SIB | + 0.572URP | − 0.177MMT | | | |
| | $R^2$ 0.605 | se | 0.721 | 0.049 | 0.104 | 0.316 | 0.102 | | | |
| | RMSE 0.413 | P | 0.045 | 0.012 | 0.010 | 0.081 | 0.094 | | | |
| | n 34 | | | | | | | | | |
| b) | MKCeff | no significant variable | | | | | | | | |
| | MKCeff | = | 0.124 | + 0.000597DUR | + 0.0277MFM | + 0.0877UKT | − 0.000987SIP | − 0.00435NFP | | |
| | $R^2$ 0.190 | se | 0.080 | 0.000 | 0.012 | 0.042 | 0.001 | 0.002 | | |
| | RMSE 0.073 | P | 0.129 | 0.052 | 0.031 | 0.043 | 0.203 | 0.057 | | |
| | n 54 | | | | | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| DUR duration (days) | n number of tests | SIB barley silage | URP urea (%) | | |
| MFM mixed feed mash | NFP neutral detergent fibre (%) | SIP silage (%) | VAC vaccinated | | |
| MMT metabolic/mode of action test | PRP crude protein (%) | UKT UK test | | | |

The Table 1 less stringent beef cattle models indicate inferior feed conversion with increased URP and improvements with increased PRP, SIB and MMT. For dairy cattle, MKCeff is enhanced with DUR, MFM and UKT and reduced in SIP rations and with higher NFP. Other dependent responses studied to date also show, for beef, that DMIeff and LWGFeff are improved with PRP and LWGeff with VAC. For dairy, other dependent response models have shown that DMIeff, MKDeff and MPPeff have regressed significantly in 1990-2003; that GFMeff, MPPeff, MFPeff and MLPeff are increased when the mixed feed component of rations is fed as mash; and that MKDeff, MPPeff, MFPeff and MLPeff are reduced with DUR. Other occasionally significant independent variables affecting responses include MMT, Holstein breed, UKT, whole cottonseed, hay (%) and total vegetable protein (%).

**Conclusions** As yet Rumzyme test data are too few to afford useful beef or dairy working models for use in practice or to model beef carcass effects. For efficient posology (dosage science), dose response researches are vitally needed. More tests are also required in order to quantify the important effects of levels of control performances, such as obtain in broilers, turkeys and laying hens. Current environmental concerns also suggest the need to test phytases and proteases in cattle nutrition.

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# Ruminal degradation characteristics of a beef feed produced with different grinding and pelleting sizes incubated *in sacco* in dry or soaked form

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**Introduction** Intensively fed ruminants receive highly fermentable diets to maximise production of meat or milk. However, highly fermentable diets increase the risk of acidosis, which can result in serious health and productive consequences. Accurate evaluation of feed fermentability in both rate and extent is therefore important in controlling acidosis. Differences in (rate of) fermentation between original raw materials are fairly well established and used in current feed evaluation systems (Van Laar et al., 2004). However, the effects of technological treatments, like grinding and especially pelleting of a feed on fermentability are less well explored. In this experiment the nylon bag method was used to analyse fermentability characteristics of a feed that was ground and pelleted in different ways. Additionally the effect on fermentability of pre-soaking the pellets prior to nylon bag incubation was investigated.

**Materials and methods** A compound feed simulating a Spanish beef feed was mixed, containing approximately 40% barley, 15 % maize gluten feed, 12 % maize, 7% wheat, sunflower meal and soya bean meal, 5% palm kernel meal and beet molasses and 2 % protected fat. This mixture was ground over a hammer mill with a 2 mm or a 3.5 mm sieve, pelleted through a die width of 3.5 or 8.0 mm, and spread on plastic on the floor to cool, resulting in 4 samples, which were stored at room temperature. Approximately 5g dry matter (DM) of each sample was weighed into nylon bags (40 µm pore size) of 10 x 20 cm. Pre-soaked (1h in 39$^{o}$C water) and un-soaked bags were incubated in the rumen of three lactating fistulated dairy cows for 0, 2, 4, 8, 16, 24, 48 and 240 hours, with 2 (2, 4 & 8h), 3 (16, 24, 48h) and 4 (240h) bags per cow. After incubation nylon bags were submersed in iced water, washed in a commercial washing machine, frozen at -18$^{o}$C, freeze dried and weighed. Bags were pooled by sample and time point for analysis of crude protein, starch and NDF. Average DM disappearance, per time point for each cow, was fitted to an exponential degradation curve: $Y = U + D*EXP(-k_d*time)$ estimating an undegradable fraction (U), potential degradable fraction (D) , and a fractional degradation rate ($k_d$) where the washable fraction (W) was calculated as: 100 – D – U, and fermentable DM as $W + D*(k_d/(k_p+k_d))$ (Ørskov and McDonald, 1979), assuming a passage rate ($k_p$) of 6 % per hour. Differences between samples in W, D, U, $k_d$ and fermentable DM were analysed as a split plot design, with cow as a random factor and the factors Grinding (G), Pelleting (P) and Soaking (S) with the MIXED procedure of SAS.

**Table 1** DM degradation characteristics for samples which have been ground (G) and pelleted (P) differently, analysed as either pre-soaked (S) or un-soaked pellets.

| G | P | S | W % | D % | U % | $k_d$ %/h | Ferm. DM % |
|---|---|---|---|---|---|---|---|
| 2 | 3.5 | Yes | 39.9 | 51.8 | 8.3 | 5.4 | 64.3 |
| 2 | 3.5 | No | 30.1 | 58.6 | 11.3 | 9.4 | 65.7 |
| 2 | 8 | Yes | 34.6 | 56.1 | 9.3 | 5.4 | 61.2 |
| 2 | 8 | No | 20.7 | 66.2 | 13.1 | 7.9 | 58.4 |
| 3.5 | 3.5 | Yes | 41.8 | 49.8 | 8.4 | 5.7 | 66.0 |
| 3.5 | 3.5 | No | 30.8 | 58.5 | 10.7 | 8.5 | 65.0 |
| 3.5 | 8 | Yes | 36.1 | 54.7 | 9.1 | 5.2 | 61.4 |
| 3.5 | 8 | No | 18.8 | 68.1 | 13.1 | 8.8 | 59.3 |
| Standard Error | | | 0.54 | 0.71 | 0.62 | 0.49 | 0.57 |
| G | | | ns | ns | ns | ns | ns |
| P | | | *** | *** | * | ns | *** |
| S | | | *** | *** | *** | *** | * |
| G*P | | | ns | ns | ns | ns | ns |
| G*S | | | * | * | ns | ns | ns |
| P*S | | | *** | *** | ns | ns | ** |

ns not significant; * P<0.05; ** P<0.01; *** P<0.001

**Results** The results for DM degradation of the 4 samples, for both un-soaked and pre-soaked analysis are in Table 1. Samples with a different grinding prior to pelleting were not significantly different in any of the degradation characteristics. The 8 mm pellets have a lower W and a higher D and U leading to a lower DM fermentability. Pre-soaked pellets had higher W and a lower D, U and $k_d$ leading to a higher DM fermentability. However there was a significant pelleting x soaking interaction, showing that soaking created larger differences in degradation characteristics for 8 mm than for 3.5 mm pellets. For the individual nutrients fermentability of pre-soaked 3.5 mm pellets was higher than for 8 mm pellets: CP (3 to 4%), starch (4 to 5%) and NDF (0 to 1%).

**Conclusions** The samples with a difference in grinding before pelleting did not differ in fermentation characteristics. A potential difference in grinding may have been obscured by subsequent pelleting. In contrast, degradation of 8 mm pellets was significantly lower than for 3.5 mm pellets, indicating that pelleting diameter affects fermentability of compound feeds. Pre soaking of pellets has a large effect on the fermentability characteristics, even interacting with pellet size. This makes the application of soaking when analyzing nylon bag degradation characteristics an important methodological consideration. When using nylon bag degradation characteristics in feed evaluation systems the effect of pellet diameter of the final compound feed should be taken into account, and may indirectly influence animal performance and health.

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# The effect of altering the plane of nutrition during different stages of the life cycle on beef cattle performance

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**Introduction** The future economic viability of beef production will require improved efficiency, reduced costs of production and ultimately cost-efficient carcass gain throughout the lifetime of the animal. The aim of the present study was to evaluate the effects of plane of nutrition during the first winter growth phase, turnout date, stocking rate, concentrate supplementation at pasture and second winter finishing phase on lifetime performance.

**Materials and methods** A total of 128 weaned, spring-born continental steer calves (mean initial live weight of 318 (sd 2.94) kg and mean age of 252 (sd 30.0) days) were allocated to a total of 32 treatments. These comprised 2 first winter growth phase x 2 turnout dates x 2 stocking rates x 2 concentrate supplementation at pasture rates x 2 second winter finishing treatments. The first winter growth phase treatments were grass silage *ad libitum* as the sole diet or supplemented with 1.5 kg concentrate/head/day fresh weight. The turnout treatments were early turnout (5th April) or late turnout (22nd April). When all cattle were turned out to pasture, animals were allocated to either high (2750 kg live weight/ha) or low (1925 kg live weight/ha) stocking rate treatment. From 1st August the cattle were allocated to two concentrate treatments either 0 or 2.5 kg concentrates/head/day fresh weight. Animals were housed on 5th October and the second winter finishing phase treatments comprised grass silage *ad libitum* supplemented with 4 kg concentrate/day fresh weight or *ad libitum* concentrate supplemented with 5 kg fresh weight silage daily. Silages offered during the first winter growth phase and the second winter finishing phases had pH and concentrations of dry matter and ammonia nitrogen (N) of 3.88 and 3.98; 168 and 309 g/kg fresh and 60 and 96 g/kg N respectively. Animals were slaughtered in 4 blocks, based on live weight at the start of the second winter finishing phase, after being offered the second winter finishing phase diet for 120, 140, 148 and 154 days. Data were analysed as a 2 (plane of nutrition during the first winter growth phase) x 2 (turnout date) x 2 (stocking rates at pasture) x 2 (concentrate supplementation at pasture from 1 August) x 2 (planes of nutrition during the second winter finishing phase) factorial design experiment using Genstat analysis of variance.

**Results** A high plane of nutrition during the first winter, early turnout and concentrate supplementation at pasture increased performance up to housing ($P<0.05$) (Table 1), but had no effect on performance during the finishing period (Table 2). Low stocking rate increased live weight at housing ($P<0.001$) (Table 1) and increased carcass weight and carcass gain at the end of the finishing period ($P<0.001$) (Table 2). A high plane of nutrition during the winter finishing period increased carcass weight, carcass gain ($P<0.001$), dressing proportion and conformation ($P<0.01$) and improved food conversion efficiency ($P<0.001$). There was a significant ($P<0.05$) interaction between plane of nutrition during the first winter and turnout date for live weight gain. At the 0 and 1.5 kg concentrate levels cattle turned out early and late had final live weights of 663, 672, 684 and 660 kg, respectively. There was an interaction ($P<0.05$) between turnout date and concentrate supplementation at pasture on live weight gain during the finishing period. For the early and late turnout treatments, receiving 0 or 2.5 kg/concentrate, live weight gains were 1.04, 0.95, 1.22 and 0.98 kg/day, respectively. There was also an interaction ($P<0.05$) between plane of nutrition during the first and second winter, on live weight gain during the finishing phase. Cattle offered 0 and 1.5 kg concentrate during the first winter and 4 kg and *ad libitum* concentrates during the second winter, had live weight gains of 0.87, 1.26, 0.93 and 1.13 kg/day, respectively.

**Table 1** The effects of altering the plane of nutrition on animal performance during the growth phase

| | First winter (W1) | | Turnout date (TO) | | Stocking rate (SR) | | Supplement (S) | | | Significance | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Low | High | Early | Late | High | Low | 0 | 2.5 | SED | W1 | TO | SR | S |
| Live weight (kg) | | | | | | | | | | | | | |
| Late turnout | 361 | 395 | 368 | 387 | | | | | 3.0 | *** | *** | | |
| 31st July | 463 | 485 | 478 | 471 | 464 | 485 | | | 4.4 | *** | NS | *** | |
| Housing | 520 | 532 | 538 | 515 | 508 | 545 | 517 | 536 | 6.1 | * | *** | *** | *** |

**Table 2** The effects of altering plane of nutrition on performance during the finishing phase and carcass quality

| | First winter (W1) | | Turnout date (TO) | | Stocking rate (SR) | | Supplement (S) | | Second winter (W2) | | Significance[1] | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Low | High | Early | Late | High | Low | 0 | 2.5 | Low | High | SED | SR | S | W2 |
| Final wt (kg) | 667 | 672 | 674 | 666 | 662 | 677 | 671 | 669 | 649 | 691 | 6.8 | * | NS | *** |
| Carcass wt[2] | 369 | 372 | 373 | 368 | 365 | 376 | 369 | 372 | 356 | 385 | 4.4 | ** | NS | *** |
| Carcass gain[3] | 0.46 | 0.47 | 0.47 | 0.46 | 0.45 | 0.48 | 0.46 | 0.47 | 0.43 | 0.50 | 0.010 | ** | NS | *** |
| Dress prop[4] | 552 | 551 | 553 | 549 | 550 | 553 | 549 | 554 | 546 | 557 | 3.5 | NS | NS | ** |
| Conf[5] | 3.32 | 3.27 | 3.36 | 3.24 | 3.36 | 3.23 | 3.30 | 3.30 | 3.17 | 3.43 | 0.087 | NS | NS | ** |
| FCE[6] | 16.9 | 16.9 | 17.4 | 16.3 | 15.9 | 17.9 | 16.0 | 17.8 | 18.8 | 14.9 | 0.92 | * | * | *** |

[1] First winter and turnout date did not ($P>0.05$) alter carcass characteristics; [2] kg; [3] kg/day; [4] Dressing proportion (g/kg); [5] Conformation based on EUROP scale where E=5 & P =1; [6]Feed conversion efficiency (kg DM intake/kg carcass)

**Conclusions** Increasing plane of nutrition at any stage of the life cycle increased animal performance during that period, however only stocking rate at pasture and plane of nutrition during the finishing period influenced carcass weight and characteristics at slaughter.

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# Effect of feeding a compound feed with a low starch content on the performance of intensively fed beef cattle

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**Introduction** The performance of Holstein Friesian bulls on commercial beef farms is highly variable and, on average, falls well short of the generally accepted cereal beef target of a minimum 260kg carcase at around a year old from cattle gaining at least 1.15kg per day from birth to slaughter (Allen and Browne 2005). The carcase weights and daily liveweight gains (DLWG) recorded by bottom 1/3rd and top 1/3rd commercial producers were 266kg and 0.95kg, and 299kg and 1.15kg respectively. It was suggested that many producers restricted concentrate feeds for at least part of the feeding period and included forage, including grazing in some cases. The formulation of the ration fed to the bulls could also have influenced performance. There is limited data on the optimum quantity of starch to include in a ration for intensively fed beef cattle The objective of this experiment was therefore to determine the effect of feeding compounds containing either a high or low level of starch to intensively reared Holstein Friesian bulls.

**Materials and methods** Thirty Holstein Friesian bulls with a mean live weight of 192kg were assigned in a randomised block design to the following dietary treatments which were fed *ad libitum*. High Starch: (Wynnstay Prime Beef, including 0.32 wheat, 0.21 barley, 0.11 rapeseed meal, 0.1 maize germ, 0.1 palm kernel and 0.05 sunflower). Low Starch: (including 0.34 sugar beet, 0.25 wheatfeed, 0.14 palm kernel, 0.11 sunflower and 0.04 rapeseed meal). The rations were formulated to be iso-nitrogenous (15% CP as fed) and iso-energetic (12.9 ME MJ/kg DM). The High Starch and Low Starch diets were analysed to contain 861 and 869 g DM, 175 and 173g CP/kg DM, 251 and 276 g NDF/kg DM, 53 and 70 g ether extract/kg DM, and 376 and 86 g starch/kg DM respectively. The cattle had free access to straw from racks and water. The cattle were housed in straw bedded yards. The bulls were selected for slaughter at MLC fat class 3. The data was analysed using ANOVA.

**Results** The bulls fed the proprietary beef nut with the high starch content recorded significantly higher slaughter weights, DLWG ($P<0.05$), carcass weights ($P<0.01$) and carcase daily gain ($P<0.001$). There was also a reduction in the number of days to reach slaughter and an improvement in feed conversion ratio (FCR) and conformation score. The number of days to slaughter was also reduced but this was not statistically significant. Despite the treatment rations being formulated to be iso-nitrogenous and iso-energetic it is suggested that the high starch ration resulted in an increased production of propionic volatile fatty acid which resulted in the improvement in animal performance. The bulls reared on the high starch diets also recorded a significantly higher ($P<0.05$) carcase price (p/kg) and carcase value ($P<0.001$).

**Table 1** Effect of compound feed starch content on animal performance

| | High Starch | Low Starch | s.e.d | Sig |
|---|---|---|---|---|
| Start weight (kg) | 193.7 | 193.0 | 11.98 | NS |
| Slaughter weight (kg) | 547.3 | 513.4 | 11.55 | * |
| Days to slaughter | 228.3 | 240.9 | 8.96 | <0.068 |
| DLWG (kg) | 1.55 | 1.33 | 0.064 | ** |
| Age (months) | 12.44 | 12.70 | 0.338 | NS |
| DLWG from birth (kg) | 1.33 | 1.21 | 0.056 | * |
| Carcase wt (kg) | 283.5 | 260.8 | 5.95 | ** |
| Kill out (g/kg) | 518 | 508 | 0.476 | NS |
| Carcase daily gain (kg) | 0.85 | 0.71 | 0.033 | *** |
| Conformation class* | 2.5 | 2.0 | 0.29 | NS |
| Fat class* | 3.0 | 2.9 | 0.23 | NS |
| Carcase price (p/kg) | 1.80 | 1.68 | 0.059 | * |
| Carcase value (£) | 510 | 438 | 12.42 | *** |

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

Table 2 Feed use (kg), Estimated FCR and Feed Costs

| | High Starch | Low Starch |
|---|---|---|
| Daily feed intake | 9.69 | 10.20 |
| Total feed intake | 2215 | 2457 |
| FCR | 6.26 | 7.69 |
| Feed cost (p/kg LWG) | 72.0 | 93.6 |

**Conclusions** The performance of the bulls fed the high starch ration exceeded the recognised targets for cereal fed beef cattle. It is suggested that the high starch ration resulted in an increased production of propionic volatile fatty acid which resulted in the improvement in animal performance. Proprietary beef nuts fed to Holstein bulls through to slaughter must be formulated to contain at least 375 g starch/kg DM. Based on the costs prevailing at the time of the study, gross margins were increased by £96 per head (from £4 to £100) with feeding the high starch ration.

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# Effect of feeding frequency on plasma insulin concentration, live weight gain and carcass composition in growing Holstein bull calves

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**Introduction** Increase of feeding frequency in cattle decreased insulin level and increased plasma glucagon concentration and consequently prevented milk fat reduction during consuming of high concentration rations (Sutton *et al*. 1986). It is also indicated that feeding of cows for six times per day instead of two times per day decreased insulin, fatty acids and butyrate level and increased GH and glucose concentration (Sutton *et al*. 1988). These changes may prevent milk fat reduction. Increased propionate concentration can stimulate insulin secretion in ruminants (Jenny *et al.,* 1972). Increased insulin secretion will stimulate glucose and fatty acid absorption and consequently will increase lipogenesis and reduce lipolysis in adipose tissue. In lactating cows, insulin usually decreases fatty acid availability for milk fat synthesis (Foster and McGarry, 2000). Previous studies have shown positive effect of increased feeding frequency on reduction of fat synthesis and deposition in body. The aim of this study was to investigate the effect of feeding frequency on insulin secretion, body weight and carcass composition in fattening Holstein bull calves.

**Material and methods** Twelve Holstein bull calves were randomly assigned in a completely randomized design (CRD). The calves were assigned to two groups according to their initial live weight and fed with total mixed ration (TMR) based on live weight individually at 2 times per day on 08:00 and 20:00 (control) or 7 times per day on 00:00, 04:00, 08:00, 12:00, 16:00, 20:00 and 24:00 (treatment). Body weight was measured at 21 days interval. Blood samples were collected 1 hour after feeding at 4 hours interval during 24 hours at the end of experiment. Moreover, four calves from each group were slaughtered and carcass compositions were measured. Plasma insulin concentrations were determined by double RIA, using validated kits (CIS bio international-Filiale de/subsidiary of Schering S.A, France). Data of live weight and plasma insulin concentration were analyzed with Proc MIXED and carcass composition weight data were analyzed with GLM procedure of SAS software.

**Results** In the present study increased feeding frequency did not have any significant effect on body weights. The results indicated that in treatment group, internal fat content was significantly ($p<0.05$) lower than those in control group (11.83±1.74 and 16.68±2.60 kg respectively). Also, depth of subcutaneous fat was significantly ($p<0.05$) higher in control than those in treatment group (1.90±0.619 and 3.32±0.194 respectively. The other carcass characteristics and parameters were not significantly different between two experimental groups. The results indicated that plasma insulin concentration were significantly ($p<0.01$) higher in control group than treatment. The results are presented in figure 1.

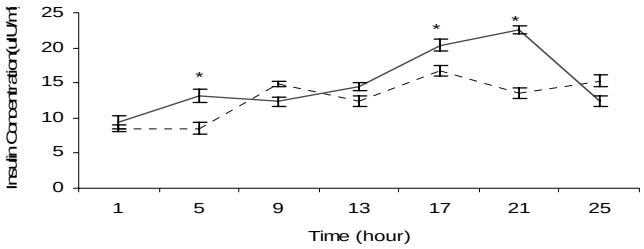


**Figure 1** Plasma insulin concentration during 24 hours at 4 hours interval in control (solid line) and treatment (dashed line).* Significant difference ($P<0.05$)

**Conclusions** In the current study, increase of feeding frequency decreased fat deposition and insulin level in Holstein bull calves. It has been suggested that reduction of insulin is due to decreased rumen propionate concentration (French and Kennely, 1995). Considering the important role of insulin in lipogenesis and reducing lipolysis (Foster and Mcgarry, 2000), it is concluded that effect of feeding frequency on body fat deposition probably is mediated by changing of insulin secretion.

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# Effects of alfalfa hay particle size and cottonseed hulls as nonforage fiber source on chewing activity and rumen pH

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**Introduction** Ration particle size has been observed to affect DMI, chewing activity, rumen pH, milk fat and nutrient digestibility (Yang et al., 2001; Grant et al., 1990). Nonforage fiber sources (NFFS) possess a large amount of NDF that can be used as a forage substitute based on their price and availability. Cottonseed hulls (CSH), a by-product of cotton processing, contain a large proportion of NDF and associated lignin, and have been considered a useful NFFS. It was suggested that physical characteristics of dietary forage can interact with nonforage fiber sources (Grant, 1997). The objectives of this study were to determine effects of CSH as nonforage fiber sources and to investigate interaction between CSH as NFFS and alfalfa particle size on Chewing Activity and rumen pH.

**Materials and methods** Twelve Holstein dairy cows in midlactation (DIM =155 ± 16; BW = 619.6 ± 54 kg) were assigned to a change-over design with three periods and 2×2 factorial arrangement of treatments. During each of 28-d periods, cows were offered one of four total mixed rations that differed in alfalfa hay particle length (long and short) and cottonseed hulls substituted for AH (0 and 9.63% DM). Dietary treatments were: long AH no CSH (LGNH), long AH with CSH (LGH), short AH no CSH (SHNH) and short AH with CSH (SHH). Long and short particle length of alfalfa hay was prepared by a farm grinder with a 10-cm or 4-cm screen, respectively. Cows were housed in tie stalls and fed twice daily at 0800 and 1400 h for adlibitum consumption. On d 26 of each period, ruminal fluid samples (approximately 100 mL) were taken with a stomach tub before morning feeding. Ruminal pH was immediately determined using a portable pH meter. On d 27 of each period, eating and ruminating activities of individual cows were visually observed and noted at 5 min intervals. Data were analyzed using the mixed model procedure of SAS (1998) with model effects for period, alfalfa hay particle length (APL), fiber source (FS) and two-way interaction of APL by FS as fixed effects and cow as random effect and effects of factors were declared at $P < 0.05$.

**Results** Dry matter intake was not affected by either APL or substitution of CSH. Decreasing APL did not affect total eating, ruminating and chewing time per day but significantly decreased eating and chewing time per kilogram NDFI ($P < 0.05$), and tended to reduced ruminating time per kilogram NDFI ($P = 0.08$). The substitution of CSH did not affect ruminating and chewing time per day tended to reduced eating time ($P = 0.06$). When chewing activity expressed as minute per kilogram NDFI or DMI decreased with the substitution of CSH ($P < 0.05$). The rumen pH was not significantly affected by either APL or the substitution of CSH.

**Table 1** Effects of alfalfa hay particle length (APL) and fiber source (FS) on eating, ruminating, and total chewing activity.

| | Treatments | | | | | Effects | | |
|---|---|---|---|---|---|---|---|---|
| | LGNH | LGH | SHNH | SHH | SEM | APL | FS | I[1] |
| Eating | | | | | | | | |
| Min/d | 263.4 | 235.1 | 242.5 | 239.6 | 11.42 | 0.32 | 0.06 | 0.12 |
| Min/kg NDF | 31.9 | 23.1 | 27.4 | 23.1 | 1.44 | ** | ** | * |
| Ruminating | | | | | | | | |
| Min/d | 479.4 | 465.6 | 468.3 | 458.9 | 14.55 | 0.3 | 0.26 | 0.83 |
| Min/kg NDF | 60.1 | 48.1 | 55.7 | 45.9 | 2.63 | 0.08 | ** | 0.53 |
| Total chewing | | | | | | | | |
| Min/d | 721.7 | 690.0 | 700.0 | 708.9 | 22.44 | 0.93 | 0.47 | 0.21 |
| Min/kg NDF | 91.8 | 71.1 | 83.1 | 69.1 | 3.51 | * | ** | 0.19 |
| Rumen pH | 6.69 | 6.72 | 6.62 | 6.69 | 0.04 | 0.11 | 0.12 | 0.48 |
| PeNDF, %[2] | 26.29 | 32.21 | 24.29 | 30.17 | 2.03 | * | ** | 0.98 |
| DMI, Kg/d | 23.48 | 25.11 | 24.94 | 25.89 | 1.17 | 0.48 | 0.21 | 0.42 |

[1]APL × FS interaction, [2] PeNDF = Ration NDF multiplied by amount of DM >1.18 mm (Kononoff et al. 2002).
*$P \leq 0.05$; **$P \leq 0.01$.

**Conclusion** These results suggest that NDF in long forage particle may be more effective in stimulating chewing activity and the NDF in CSH may be less effective in stimulating chewing activity than the NDF in both particle length of AH used in this study. In addition, alfalfa particle size is a poor predictor of total chewing time and rumen pH.

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# Effects of lucerne hay and barley grain particle sizes on chewing activity, rumen pH and milk composition of Holstein dairy cows

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**Introduction** NRC (2001) recommended 250 g/kg dietary neutral detergent fibre (NDF) with a minimum of 190 g/kg dietary forage NDF to prevent milk fat depression. This recommendation does not account for the differences in forage particle size. The forage particle size requirements of dairy cows fed barley grain-based diets can differ from those fed maize grain-based diets (Yang and Beauchemin, 2006). The objective of this experiment was to evaluate the effects of lucerne hay and barley grain particle sizes on chewing activity, rumen pH, milk yield and milk composition using barley grain-based diets.

**Material and methods** Eight multiparous Holstein dairy cows in early lactation (40±10 DIM) with 45±5 kg/d milk yield were used in a 2×2 Latin square experiment with four 21-d periods. Factorial arrangement of treatments consisted of two levels of lucerne hay particle size (954 vs. 544 g/kg of DM was >1.18 mm for long (LL) and short (SL) lucerne, respectively) and two levels of barley grain particle size obtained with a hammer mill using two screens (2.5 mm as coarse (CB) and 1.5 mm as fine (FB) barley). Diets were formulated based on NRC (2001) and offered as TMR twice daily. Particle size distribution was determined according to ASAE (1988, 1992). Milk samples were analyzed for fat, protein and lactose (MilkoScan Conveyor 4000, Foss Electric, Denmark). Rumen liquor was taken by stomach tube prior to morning feeding and 2 h post-feeding in d 19 and at 3 and 4 h post-feeding in d 20 of each period. Chewing activity was recorded using a digital camera and a computer.

**Results** Reducing lucerne particle size (LPS) reduced physically effective NDF based on 1.18 mm sieve ($peNDF_{1.18}$) from 227 to 204 g/kg DM of TMR and reduced DM intake (P<0.05, Table 1). Ruminating activity as according to all the evaluated indices was reduced with LPS reduction (Table 2). Reducing barley grain particle size (BPS) increased ruminating activity expressed as min/kg forage NDF and $peNDF_{1.18}$. Total chewing activity decreased by reducing LPS according to all the indices but increased by reducing BPS when expressed as min/kg $peNDF_{1.18}$. Fat corrected milk yield (4%) decreased by reducing LPS. Milk fat concentration and yield decreased by reducing LPS. Milk protein concentration and yield increased by reducing both LPS and BPS. Rumen pH at 0, 2, 3 and 4 h post feeding decreased by reducing BPS but it decreased by reducing LPS only at 3 and 4 h post feeding.

**Conclusion** The fermentability of dietary concentrate affected rumen pH more than forage particle size. In order to maintain rumen pH, milk fat and animal health, physical and chemical properties of the concentrate in addition to physical and chemical properties of the forage should be considered.

**Table 1** Dry matter intake, milk yield and milk composition of dairy cows

| | LL | | SL | | | | | |
|---|---|---|---|---|---|---|---|---|
| | CB | FB | CB | FB | SEM | L[1] | B | L*B |
| DM intake, kg/d | 27.1 | 26.1 | 24.7 | 25.9 | 0.52 | * | ns | * |
| Milk yield, kg/d | 40.5 | 40.6 | 40.8 | 41.4 | 0.48 | ns | ns | ns |
| FCM yield, kg/d* | 36.5 | 36.2 | 35.6 | 35.3 | 0.37 | * | ns | ns |
| Milk fat, g/kg | 33.4 | 32.8 | 31.4 | 30.2 | 1.2 | * | ns | ns |
| Milk fat , g/d | 1353 | 1331 | 1282 | 1249 | 38 | * | ns | ns |
| Milk protein, g/kg | 31.4 | 32.6 | 32.9 | 34.1 | 0.5 | * | * | ns |
| Milk protein, g/d | 1272 | 1323 | 1343 | 1398 | 25 | * | * | ns |

* 4% fat corrected milk yield, [1]L=effect of lucerne hay particle size,
B = effect of barley grain particle size, ns = non significant, * = p<0.05

**Table 2** Ruminating and total chewing activities of dairy cows

| | LL | | SL | | | | | |
|---|---|---|---|---|---|---|---|---|
| | CB | FB | CB | FB | SEM | L[1] | B | L*B |
| Ruminating | | | | | | | | |
| min/d | 426 | 468 | 335 | 367 | 48.2 | * | ns | ns |
| min/kg DM | 15.7 | 18.0 | 13.5 | 14.2 | 1.45 | * | ns | ns |
| min/kg total NDF | 51.6 | 58.9 | 44.5 | 46.5 | 3.44 | * | ns | ns |
| min/kg forage NDF | 89.9 | 102.6 | 77.5 | 81.1 | 4.02 | ** | * | ns |
| min/kg $peNDF_{1.18}$ | 62.4 | 74.2 | 57.2 | 62.2 | 3.24 | * | * | ns |
| Total Chewing | | | | | | | | |
| min/d | 712 | 742 | 573 | 615 | 58.3 | * | ns | ns |
| min/kg DM | 26.3 | 28.5 | 23.2 | 23. 8 | 1.84 | * | ns | ns |
| min/kg total NDF | 86.2 | 93.3 | 76.0 | 78.0 | 4.73 | * | ns | ns |
| min/kg forage NDF | 150.3 | 162.7 | 132.5 | 135.9 | 5.29 | * | ns | ns |
| min/kg $peNDF_{1.18}$ | 104.3 | 117.6 | 97.9 | 104.3 | 4.19 | * | * | ns |

[1] L= effect of lucerne hay particle size, B = effect of barley grain particle size
ns = non significant, * = p<0.05, ** = p<0.01

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# Microbial-N supply and milk yield cows in a silvopastoral system with and without access to the forage tree and energy supplementation during the dry season

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**Introduction** Supplementation with legume fodder during the dry season is a strategy to improve animal performance. The results obtained in silvopastoral systems has been explained either by their ingestion of a higher quality grass (Hernandez et al., 2001, Iglesias, 1998) resulting from the inclusion of a legume in the system which provide N for grass growth or due to the intake of the legume itself, which in turn is also of higher quality than grass (Kakengi et al., 2001), and provides a higher microbial-N supply (Karda and Dryden, 2001). The objective of the present work was to discriminate, in a silvopastoral system, the effect of improved grass quality and legume intake (*L. leucocephala*) on rumen fermentation, microbial N supply, milk yield and composition of dual purpose lactating cows.

**Material and methods** Four crossbred cows (476 ± 49kg LW) fitted with rumen cannula were used in latin square design to study NH-N, VFA's, and microbial-N supply (total urine collection and urinary purine derivatives determination). Eight crossbred cows (508 ± 53 kg LW, without cannula) were used in a latin rectangle design to study milk yield and composition. Cows were allocated to each treatment during each one of the four 15 d experimental period (10 d adaptation and 5 d sampling). Treatments were: Grass= Guinea grass (Panicum maximum) monoculture. SP-L= silvopastoral system (grass + leucaena) but cows did not have access to Leucaena fodder (it was harvested before the cows went into the paddock), SP= silvopastoral system (cows had access to Leucaena fodder), SP+M= silvopastoral system + energy supplementation (maize grain, 0.4% LW). Cows were kept in a 42 d rotational grazing system with 1 d occupation period. Paddock size was adjusted to provide 24 kg DM.animal $d^{-1}$. Cows were milked once a day in the morning and during the sampling period 4 I.U. oxytocin was used to ensure complete milk extraction. Preplanned orthogonal contrast of interest were, C1= Grass vs SP-L, C2= SP-L vs SP and C3= SP vs SP+M.

**Table 1** Chemical composition (g.kg $DM^{-1}$) of the grass in the different grazing system and of L. leucocephala fodder.

| | Grass | GSP-L | GSP | L |
|---|---|---|---|---|
| OM | 889 | 884 | 884 | 928 |
| CP | 55 | 54 | 53 | 203 |
| NDF | 729 | 715 | 709 | 398 |
| ADF | 402 | 402 | 406 | 242 |
| Lignin | 58 | 69 | 72 | 116 |

Grass= grass only, GSP= grass in the silvopastoral system GSP-L= GSP without access to leucaena fodder, L= L. leucocephala fodder

**Results** No major changes were observed in the quality of the grass in the silvopastoral systems as compared with the grass monoculture (Table 1). Access to Leucaena increased rumen $NH_3$ concentration, urinary purine derivatives excretion and microbial nitrogen supply, which were further improved with energy supplementation but did not change milk yield and major milk constituents. Milk urea increased with leucaena intake but decreased when an energy supplement was offered (Table 2).

**Table 2** Mean $NH_3$-N (mg.$L^{-1}$), VFA's (mmol.$L^{-1}$), Purine derivatives (mmol.$d^{-1}$) milk yield (kg.$d^{-1}$) and composition (g.kg $Milk^{-1}$, except milk urea mg.$dL^{-1}$) in cows under different system.

| | Grass | SP-L | SP | SP+M | SEM | C1 | C2 | C3 |
|---|---|---|---|---|---|---|---|---|
| $NH_3$ | 37.5 | 47.7 | 68.6 | 59.1 | 4.54 | NS | * | NS |
| VFA's | 67.7 | 73.2 | 73.5 | 77.1 | 3.09 | NS | NS | NS |
| Total PD | 90.7 | 102.5 | 118.6 | 133.1 | 4.85 | NS | * | NS |
| Microbial N | 60.5 | 70.3 | 93.2 | 110.4 | 5.5 | NS | * | * |
| Milk Yield | 8.2 | 8.1 | 8.5 | 9.3 | 0.7 | NS | NS | NS |
| Lactose | 57.3 | 54.3 | 58.9 | 58.1 | 2.6 | NS | NS | NS |
| Fat | 30.9 | 32.5 | 33.6 | 33.5 | 2.4 | NS | NS | NS |
| Protein | 30.2 | 30.3 | 30.5 | 31.9 | 0.9 | NS | NS | NS |
| Urea | 17.1 | 17.2 | 24.0 | 20.3 | 1.52 | NS | * | * |

Grass= grass only, SP= silvopastoral system
SP-L= SP without access to Leucaena
SP+M= SP with maize grain supplementation
C1= grass vs SP-L, C2= SP-L vs SP, C3= SP vs SP+M

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**Conclusions** When a low quality grass is available as during the dry season, access to leucaena and energy supplementation can improve rumen environment and increase microbial-N supply. However, except for MUN, milk yield and composition were not affected.

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# Predicting energy and protein supply and milk production of dairy cows consuming high forage rations in the central highlands of Kenya

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**Introduction** Level 1 of the Cornell Net carbohydrate and Protein System (CNCPS) model has not yet been used to predict animal performance on smallholder dairy farms in African conditions. Over 60% of the smallholder dairy cattle in sub-Saharan Africa are in the East African highlands. Although the level of production is low, dairy cattle contribute significantly to the livelihoods of rural families. If the model predictions reflect animal performance, the CNCPS model could be a useful tool to develop feeding strategies to enhance animal production. The objectives of our study were to: (1) evaluate the accuracy of the predictions of dry matter intake, milk production and phosphorus excretion of the CNCPS model for dairy cattle on smallholder farms; (2) predict nutrient requirements and supply of confined and lactating crossbred cows consuming high forage rations and (3) determine the variation in feed quality and milk production.

**Material and methods** Twenty-four farms with a total of 26 crossbred lactating cows were randomly selected for collection of animal and feed data. The cows were confined in zero-grazing pens that were partially roofed and had both feed and water troughs; calves were housed separately. Lactating cows were fed whole or coarsely chopped forage in the morning while concentrate feed (when available) was provided during milking. Cows were hand milked twice daily before 1000 h and after 1600 h and milk weights were recorded at each milking. Animal production and feed data, as well as data on management and the environment were measured monthly. The data were collected from September 2003 until August 2004 and used in predicting nutrient requirements, concentration of nutrients in dry matter intake (DMI), balance of Metabolizable protein and metabolizable energy and of Ca and P; milk production and milk phosphorus (P) secretion, and fecal P excretion (Fox et al., 2004). Model predictions of DMI, milk production and P secretion were evaluated against observed data using the Model Evaluation System software (MES, v. 2.0) (Tedeschi, 2006). Seasonal variation in diet concentration, nutrient balances (ME, MP, Ca and P) and milk production were also assessed using the ANOVA procedures of Minitab (Release 14.13)

**Results** Napier grass (*Pennisetum purpureum*) and stems and leaves of banana (*Musa sapientum*) were important forages in the rainy seasons while maize (*Zea mays*) stover was fed during the dry seasons. Dry matter intake averaged 8.4 kg/d and forage NDF intake was 5.5 kg/d; about 1.6 % of live weight. Intake of commercial concentrates was low. Lignin content of long rainy season rations was 8.5 % of DM and ME levels were correspondingly lower (1.7 Mcal/kg) than in other seasons. Dietary energy was inadequate to meet the requirements for most cows and greater deficits were evident in rations of high producing cows (Table1). Rumen nitrogen balance was positive, but energy supply was low resulting in low microbial protein production.

**Table 1** Diet energy and protein balance and performance of early lactation cows consuming forage diets.

| | Low milk cows | | | | | High milk cows | | |
|---|---|---|---|---|---|---|---|---|
| Parameter/season | LongDry | ShortRains | ShortDry | LongRains | Stdev | ShortRains | LongRains | Stdev |
| Observations | 12 | 13 | 8 | 8 | | 4 | 5 | |
| Body weight (kg) | 294 | 322 | 315 | 320 | 41.0 | 505[a] | 401[b] | 34.0 |
| Actual Milk (kg/d) | 6.6 | 5.6 | 6.7 | 4.4 | 2.2 | 15.2 | 18.1 | 1.2 |
| ME milk (kg/d) | 4.8 | 3.1 | 4.4 | 3.2 | 2.0 | 10.2 | 12.5 | 0.4 |
| ME balance Mcal/d | -2.2 | -3.2 | -2.1 | -1.2 | 2.0 | -5.0 | -5.8 | 1.9 |
| MP balance (g/d) | -41.4 | -20 | -34 | 12.3 | 96.0 | -55 | -25 | 99.2 |
| ME (Mcal/d EBW) | 1.8 | 1.9 | 2.2 | 1.4 | 0.9 | 5.6 | 5.1 | 1.2 |

ME = metabolizable energy Mcal/d; MP= metabolizable protein; EBW = Empty body weight; stdev = standard deviation.
[a,b] Means within a row without a common superscript differ ($P < 0.05$)

The CNCPS predicted values of DMI for all lactating periods were close to observed values ($r^2 = 0.71$, mean bias = -0.24 kg/d), but DMI during early lactation was over-estimated by 0.81 kg/d. Prediction of milk production was also accurate, with $r^2 = 82$ % and model bias of -2.2 %. Predictions of milk and manure P were less accurate: systematic bias was 10 and 11.1 %, respectively.

**Conclusion** There is need to fully characterize common tropical feeds to improve the accuracy in prediction of feed biological values and aid designing of feeding strategies that meet requirements of cows on smallholder farms using tools as the CNCPS.

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# Nutrients digestion and milk production of Holstein dairy cows fed various amounts of soybean hulls

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**Introduction** Feed costs account for large part of the total costs in milk production. Thus, dairy producers attempt to minimize the costs of feeding their herds particularly when milk prices are low. By-product feeds such as soybean hulls, In addition to the potential of providing an economical alternative, can be successfully used as a source of fiber in rations for dairy cattle when forages have poor quality or are in short supply (Firkins, 1997; Weidner and Grant, 1994). Alternatively, replacing cereal grains with SH in diets for dairy cows may contribute to elevated intakes of energy while preventing a disruption of rumen functionality (Ipharraguerre et al., 2003). The objectives of this paper are to evaluate effects of soybean hulls in replacement of alfalfa hay, corn silage and wheat bran on performance and total tract apparent nutrients digestibility.

**Materials and methods** Twelve mono and multiparous midlactation Holstein dairy cows, used in a change-over design in order to evaluate the effects of soybean hulls on milk production and composition, digestibility of nutrients, chewing activity and rumen pH. Diets fed ad libitum two times in day (0800 and 1400) as a total mixed rations and consisted of 0, 10, 20 and 30% of soybean hulls, respectively. In experimental diets, Soybean hulls replaced with alfalfa hay, corn silage and wheat bran in same amounts. Dry matter intake and Milk production measured daily and milk composition measured twice in week. Rumen pH and chewing activity measured in the end of each period. Digestibility of nutrients measured using acid insoluble ash as marker. Comparison of least square means between each treatment was carried out using Mixed procedure of SAS (SAS 1996). The model was as following: $Yij = \mu + Ti + Pj + Cowk + eijk$ that Yij is the observations, $\mu$ is the overall mean, Ti is the treatment effect, Pj is the period effect, Cowk is the cows' effect and eijk is the residual effect.

**Results** Dry matter intakes decreased significantly when soybean hulls used in high amounts in diets, and increased when it was used in low amounts ($P<0.05$). Production of Milk and 4% fat-corrected milk, milk fat percentage and yield, milk crude protein percentage and yield, did not significantly differed among treatments. Total tract apparent digestibility's of NDF and NFC, increased and decreased respectively in high (20 and 30%) soybean hulls diets ($P<0.01$). Diets did not affect total eating, ruminating and chewing activities (min/day). But, ruminating and chewing activities for kg intake of NDF and peNDF, showed significant difference among diets. High soybean hulls diets had lower chewing activity per kg intake of NDF and higher for chewing per kg of peNDF intake ($P<0.01$). However, body weight gain and total tract apparent digestibility's of DM, OM and CP were similar among diets. There was a significant difference in rumen pH between high soybean hulls contained diets with 10% soybean hulls and control diets ($P<0.05$).

**Table 1** Effects of control and soybean hulls diet on cows' performance and nutrients digestibility.

| | Percentage of soybean hulls in diets (DM basis) | | | | | |
|---|---|---|---|---|---|---|
| | 0% | 10% | 20% | 30% | SEM | *P* |
| Dry matter intake kg/d | 21.4 a | 22.2 a | 18.8 b | 19.5 b | 0.94 | * |
| Milk Yield (kg/d) | 25.9 | 26.2 | 25.2 | 26.3 | 1.7 | NS |
| Milk Protein (%) | 2.89 | 2.95 | 3.04 | 3.09 | 0.175 | NS |
| Milk Fat (%) | 3.45 | 3.42 | 3.48 | 3.46 | 0.24 | NS |
| Digestibility of DM (%) | 65.02 | 64.84 | 64.97 | 64.72 | 0.75 | NS |
| Digestibility of CP (%) | 65.4 | 63.9 | 63.6 | 63.1 | 1.7 | NS |
| Digestibility of NDF (%) | 43.1 c | 47 b | 51.6 a | 50.8 a | 1.04 | ** |
| Digestibility of NFC (%) | 91.9 a | 90.4 ab | 87.1 b | 87.6 b | 1.42 | ** |
| Rumen pH | 6.8 a | 6.75 ab | 6.6 b | 6.6 b | 0.046 | * |

**Conclusion** The results show that soybean hulls is a useful alternative feedstuff for lactating Holstein cows without any undesirable effects on performance, milk production and nutrients digestion. Then it can be successfully used as replacement of concentrate fractionin or a source of fiber for dairy cattle when forages have poor quality or are in short supply.

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# Effect of substitution of soybean meal by dried tomato pomace on feed intake, rumen fermentation and nitrogen utilisation in goats

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**Introduction** Goat production in northeast of Thailand has gradually increased, partly because of the limited land available to raise cattle. The other reason is that goats can give twin kids and can give birth twice a year under good management. Tomato pomace is a by product from the tomato juice factory. Dried tomato pomace is a promising protein source with high protein content. The price of dried tomato pomace is cheaper than soybean protein when expressed as price per unit protein. The trial of Yuangklang et al. (2006) found that dried tomato pomace increased growth rate in beef cattle. In dairy cows, replacing soybean meal by dried tomato pomace did not influence milk production (Yuangklag et al., 2005). Data on utilisation of dried tomato pomace in goats is limited. Therefore the present experiment aimed to investigate the effects of substitution of soybean protein by dried tomato pomace in the concentrate diet on feed intake, nutrient digestibility and nitrogen utilization of meat goats.

**Materials and methods** Four crossbred goats were used in a 4x4 Latin square experiment with 4 21days periods. Treatments were varying soybean (SBM) to dried tomato pomace (DTP) ratios in the concentrate as follows; 100%SBM, 75%SBM:25%DTP; 25%SBM:75%DTP and 100%DTP. Fresh Napier grass was offered ad lib. Concentrate was offered at 1.5%BW. During the last 5 days of each period, total collection was performed to determine nutrient utilisation. Diet and faeces samples were analyzed according to standard procedures. Rumen fluid was collected on the last day of each period and measured for pH and $NH_3$-N.

**Results** Roughage and total intakes were similar for all treatments. Concentrate intake was slightly decreased as DTP inclusion increased ($P>0.05$). Ruminal pH and ammonia nitrogen were linearly increased ($P<0.05$) as DTP increased. This result might be due to the high rumen degradability of DTP . This would increase nitrogen accumulation in the rumen, leading to an increase in rumen pH. N intake, urinary N, N balance, N retention and N absorption did not differ significantly between treatments ($P>0.05$), but faecal N increased linearly as DTP inclusion increased ($P>0.05$). It is possible that nitrogen could not be captured and utilized within the rumen so that nitrogen is excreted in the faeces.

**Table 1** Effect of varying the Soybean meal:dried tomato pomace inclusion ratios on feed intake, rumen pH, rumen $NH_3$-N and nitrogen utilisation.

| | SBM:DTC ratios in concentrates | | | | | P-value | | |
|---|---|---|---|---|---|---|---|---|
| | 100:0 | 75:25 | 25:75 | 0:100 | SEM | L | Q | C |
| Intake, gDM/d | | | | | | | | |
| Roughage | 530 | 534 | 536 | 535 | 7.88 | ns | ns | ns |
| Concentrate | 330 | 325 | 322 | 320 | 2.55 | ns | ns | ns |
| Total | 860 | 859 | 858 | 855 | 7.90 | ns | ns | ns |
| pH | 6.21 | 6.39 | 6.47 | 6.56 | 0.06 | * | ns | ns |
| $NH_3$-N, mg% | 18.23 | 19.34 | 19.89 | 20.21 | 0.46 | * | ns | ns |
| N intake, g/d | 18.7 | 18.6 | 18.5 | 18.5 | 0.13 | ns | ns | ns |
| N in feces, g/d | 3.35 | 3.41 | 3.49 | 3.55 | 0.08 | ns | ns | ns |
| N in urine, g/d | 0.45 | 0.44 | 0.46 | 0.44 | 0.03 | ns | ns | ns |
| N balance, g/d | 14.9 | 14.8 | 14.6 | 14.5 | 0.06 | ns | ns | ns |
| N retention, g/d | 15.4 | 15.2 | 15.0 | 15.0 | 0.04 | ns | ns | ns |
| N absorption, % of intake | 82.4 | 81.7 | 81.1 | 81.1 | 1.25 | ns | ns | ns |

*$P<0.05$

**Conclusions** From the present experiment it would appear that dried tomato pomace can replace soybean meal in the diet of the goat without detriment to feed intake or nitrogen utilization. The study should be followed by production trials looking at the influence of DTP on growth, feed conversion and carcass attributes in goats

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# Estimation of mature live weight of Mexican Pelibuey sheep

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**Introduction** Current nutritional models as the CNCPS-sheep (Cannas et al., 2004) are built with the possibility to consider breed effects. An important effect included in the prediction of live weight gain is the maturity index of the animal (the actual weight of the animal in relation to their adult weight). During a concurrent evaluation of the CNCPS-sheep model, no information was found on the Pelibuey sheep mature weight to allow a proper adjustment of this parameter. Thus the objective of this work was to estimate the mature weight of Pelibuey sheep.

**Materials and methods** Historical data on age and weight of both males and female pelibuey sheep were collected from four farms. A total of 3320 data were collected for males and 1857 for females. The data were fit with a non linear function $Y_t = A( 1 - Be^{-kt})$ minimizing the sum of squares or relative distances (relative weighting or weighting by $1/y^2$) (Motulsky and Christopoulos, 2003) to provide estimates of rates of maturing (k) and mature weight (A) which were the main values of interest (Jenkins and Leymaster, 1993). Parameters of the monomolecular growth function have been previously described in biological terms (Brody, 1945). Data were fit and parameters compared with a "t test" using the GraphPad Prism 4 Software (Graph Pad Prism 1994-2003).

**Results** Estimated mature weight of both male and female pelibuey sheep is presented in Table 1. Growth pattern is presented in Figure 1. Males were heavier at mature weight and grew faster than females ($P<0.05$).

**Table 1** Parameters estimates (± S.E.) characterizing growth of live weight of Pelibuey sheep.

| Parameters | Males | Females |
|---|---|---|
| A (mature weight) | 65.8[a] ± 2.474 | 45.13b ± 1.83 |
| B (constant of integration) | 0.965[a] ± 0.001339 | 0.9518b ± 0.00204 |
| K (maturing rate) | 0.002838[a] ± 0.0001348 | 0.002392b ± 0.0001226 |
| 95% Confidence Intervals | | |
| A | 60.95 to 70.65 | 41.54 to 48.72 |
| B | 0.9624 to 0.9676 | 0.9478 to 0.9558 |
| K | 0.002573 to 0.003102 | 0.002152 to 0.002633 |
| Goodness of Fit | | |
| Degrees of Freedom | 3317 | 1854 |
| $R^2$ (unweighted) | 0.7931 | 0.8578 |
| Weighted Sum of Squares ($1/Y^2$) | 295.9 | 188.4 |
| Absolute Sum of Squares | 141778 | 24094 |
| Sy.x | 6.538 | 3.605 |

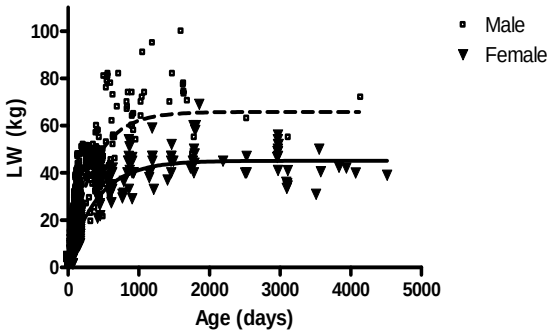


**Figure 1** Growth pattern of pelibuey sheep

**Conclusions** Mexican Pelibuey sheep male mature weight was estimated as 65 kg while the ewe mature weight was 45 kg.

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# Estimation of genetic and phenotypic parameters for fleece characteristics of Sanjabi sheep breed

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**Introduction** Sanjabi breed is an important dual-purpose (meat and wool) sheep and one of the heavy weight and the second largest populated of sheep among 27 breeds in Iran. According to FAO (2004) there are more than 53 millions sheep are reared in Iran. The breed is well adapted to reasonably suitable environment, vegetation and management conditions of the western part of Iran. In the western part of Iran although there are rocky mountains, there is enough feed source for raising sheep. The objective of this study was to estimate genetic parameters for planning sustainable improvement, conservation and utilization of the breed.

**Materials and methods** A total of 227 records of greasy fleece weight (GFW), staple length (SL) and body weight (BW), and 163 records of fibre length (FL), true wool percentage (TWP), medullated fibre percentage (MFP), kemp fibre percentage (KFP) and wool wax percentage (WWP) were measured according to Dashab et al. (2006) in order to estimate genetics and phenotypic parameters. Due to unequal sub class numbers, the data was analysed using the method of least squares as outlined by Harvey (1987). Genetic and phenotypic correlations were estimated through analysis of covariance of parental half-sibs using Harvey's LSMLMW program (1987). The mean and standard deviation of considered traits are presented in Table 1.

**Results** Body and greasy fleece weights were highly significantly affected by age ($P<0.01$). While sire effects had a highly significant fluctuation on BW, GFW, SL and WWP ($P<0.01$). Greasy fleece weight was about 2.8 kg compare to average of sheep fleece production in Iran reported by British Wool Statistics for year 2004 (1.1 kg). There were strong heritability for BW and GFW (0.53 and 0.77, respectively) and also genetic and phenotypic correlations between BW and GFW were positive and high (0.63 and 0.59, respectively) for effective selection (Table 1). Only 65 percent of the fibres were true wool and the rest were medulated fibres and kemp fibres (18.5 and 16.5 percent, respectively). Among fibres 64.5 percent of them had the diameter less than 28.75 micrometer, while 33.5 percent of the fibres had the diameter between 28.75 and 48.75. Only 4 percent of the fibres had the diameter higher than 48.75 micrometer.

**Table 1** Least-squares means±SE of the considered traits and also heritability±SE (diagonal, underline), genetic (above diagonal) and phenotypic (below diagonal) correlation for Sanjabi sheep

| Traits | BW (kg) | GFW (kg) | SL (cm) | FL (mm) | TWP (%) | MFP (%) | KFP (%) | WWP (%) |
|---|---|---|---|---|---|---|---|---|
| LS-mean | 42.6±8.5 | 2.8±0.6 | 13.1±2.1 | 78.6 ±23 | 65.0±4.1 | 18.5±6.5 | 16.5±2.1 | 3.0±0.5 |
| BW | 0.53±.2 | 0.63 | 0.45 | 0.53 | 0.51 | 0.35 | 0.18 | -0.38 |
| GFW | 0.59* | 0.77±.3 | 0.3 | 0.16 | 0.85 | 0.4 | 0.31 | -0.4 |
| SL | 0.36* | 0.47* | 0.47±.2 | 0.57 | 0.78 | 0.02 | -0.36 | -0.22 |
| FL | 0.19* | 0.24* | 0.50* | 0.37±.2 | 0.63 | 0.31 | -0.22 | -0.36 |
| TWP | 0.03 | 0.10 | -0.01 | -0.04 | 0.35±.2 | -0.59 | 0.22 | 0.57 |
| MFP | -0.04 | 0.10 | 0.02 | 0.03 | 0.01 | 0.35±.2 | 0.52 | -0.57 |
| KFP | 0.01 | 0.03 | 0.05 | -0.78* | -0.23* | 0.09 | 0.24±.2 | -0.61 |
| WWP | -0.12* | -0.14* | -0.11* | 0.06 | 0.02 | -0.07 | 0.11 | 0.83±.3 |

* Significant at ($P< 0.05$)

**Conclusions** Greasy fleece weight of this breed is 2.5 times the national average of fleece production for sheep shows the good potential of this breed for wool production programs. Strong heritability for body weight and greasy fleece weights also shows this breed could be improved through individual section. Also high positive genetic and phenotypic correlation between these two traits shows selection for any of them would increase both traits at present genetic makeup of the breed. Combination and average fibre diameter of the breed made the fleece suitable for carpet industry.

**Acknowledgements** Supports of Isfahan University of Technology is gratefully acknowledged.

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# Analysis of variance on economic traits of Karakul sheep for determination selection index

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**Introduction** The selection index is a method for estimation the breeding value of an animal combining all information available on the animal and its relatives. Research shows that genetic and phenotypic correlation between traits is completely different among breeds. In baluchi sheep, genetic and phenotypic correlation between weaning weight and yearling weight are 0.93 and 0.59 respectively. Another study on hamshire sheep shows that correlation between birth weight and yearling weight is 0.48 and 0.23. The objective of this study is to predict different type of correlation in karakul sheep traits for determination selection index.

**Materials and methods** For determination selection index these traits were used: birth weight (BW), three month weight (3W), nine month weight (9W) and yearling weight (YW). 3430 records of karakul sheep were used. Fixed effects that influence these traits (sex, birth type, birth year, birth month and ewe age) analyzed with JMP4.0 under multiple regression method. Characteristics of records are given in Table 1. All bi-variant analysis did with model of DXMUX in DFREML and additive genetic effect considers as a random effect on traits. Genetic parameters were estimated by using REML method under animal model. The best used models were model I; Y=Xb+Za+e and model II; Y =Xb+Za+Wp+e. In these models Y is vector of observation, b is vector of fixed effect; a is vector of additive genetic effect and X, Z and W are matrices due to fixed and random effect and e is vector of residual effect. We Assumed that birth weight (BW) and three month weight (3W) are selection criterion but nine month (9W) and yearling weight (YW) are selection objective. Established P, C and G matrices and estimated selection index coefficient with GENUP software under this equation: $b=P^{-1}Ga$.

**Table 1** Characteristics of records

| Character | BW | 3W | 6W | 9W | YW | ADG BW-3W | ADG3W-YW |
|---|---|---|---|---|---|---|---|
| Mean *kg* | 5.15 | 24.15 | 32.50 | 39.44 | 43.62 | 0.211 | 0.078 |
| Standard deviation *kg* | 0.79 | 5.04 | 5.40 | 6.72 | 7.58 | 0.048 | 0.023 |
| Coefficient of variation % | 15.33 | 2.087 | 16.61 | 17.04 | 17.37 | 22.74 | 29.48 |
| Number of records | 3430 | 2986 | 2837 | 2346 | 2474 | 2870 | 2440 |
| Number of fixed effect | 4 | 4 | 5 | 4 | 5 | 4 | 4 |
| Best model | II | II | I | I | I | II | I |

**Table 2** Parameters resulted from bi-variant analysis

| Trait1 | Trait2 | $h^2$1 | $h^2$ 2 | rg | rp |
|---|---|---|---|---|---|
| BW | 9W | 0.34 | 0.31 | 0.14 | 0.25 |
| BW | YW | 0.33 | 0.31 | 0.29 | 0.21 |
| 3W | 9W | 0.38 | 0.32 | 0.78 | 0.54 |
| 3W | YW | 0.42 | 0.31 | 0.85 | 0.55 |
| ADG BW-3W | ADG 3W-YW | 0.37 | 0.14 | 0.39 | -0.17 |
| 9W | YW | 0.39 | 0.3 | 0.88 | 0.72 |

$h^2$1= trait 1 heritability; $h^2$ 2= trait 2 heritability; rg = genetic correlation , rp=phenotypic correlation

**Results** Parameters resulted from bi-variant analysis are in Table 2. In this study additive genetic correlation between different weights are positive and the other research show these results. Phenotypic correlation between ADG bw-3w and ADG3w-Yw is negative. Research indicate that Genetic correlation between weight traits is high so we can used from weight record in lower ages to select lambs for future. Co (variance) and other parameters for making selection index were used from Table 3. Selection index that calculated is $I=-18.46X_1+ 49.49X_2$ that $X_1$ and $X_2$ are birth weight and three month weight respectively.

**Table 3** Parameters for making matrices P, C and G

| | BW | 3W | 9W | YW | $\delta^2$p | a |
|---|---|---|---|---|---|---|
| BW | 0.26 | 0.36 | 0.25 | 0.21 | 0.44 | - |
| 3W | 0.45 | 0.2 | 0.54 | 0.55 | 15.31 | - |
| 9W | 0.14 | 0.78 | 0.29 | 0.72 | 21.73 | 100 |
| YW | 0.29 | 0.85 | 0.88 | 0.3 | 23.84 | 100 |

$\delta^2$p= phenotypic variance; a= economic value (as a correction factor)
Heritability is on diagonal. Phenotypic and genetic correlation are above and below of diagonal respectively.

**Conclusion** We can select male and female lambs in month 3. Initially, all lambs with genetic disorders should be culled and then, after putting birth and three month weight into the selection index formula, an index for every lamb can be calculated. They can then be selected according to these indices.

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# Toxoplasma gondii in sheep: Epidemiological clues from wild rabbits and hares

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**Introduction** The protozoan parasite *Toxoplasma gondii* is an important cause of lamb loss. Horizontal transmission to ewes can occur from the parasite's definitive host, the cat, by the faecal-oral route (Buxton 1990). Vertical (transplacental) transmission to lambs also occurs (Williams *et al* 2005; Rodger *et al* 2006). *Toxoplasma* encysts in brain and muscle where it persists for the lifetime of the sheep. Since *Toxoplasma* can infect any warm-blooded vertebrate, wild rabbits (*Oryctolagus cuniculus*) and hares (*Lepus europeaus*) might similarly be infected by the faecal-oral route and they might function as parasite reservoirs, infecting cats by the tissue-oral route. The aim of this study was to determine whether rabbits and hares are informative as sentinel species, revealing the prevalence and strain of *Toxoplasma* on pasture, such that they could be used as indicators of infection risk to sheep.

**Materials and methods** The heads of 100 rabbits and 10 hares shot at sites in North Yorkshire in August 2006 were vacuum packed and stored at -20°C. For hares, blood samples were collected during butchering and stored at -20°C. For rabbits, dried blood was recovered from the vacuum bags by rinsing with 0.85% saline. Blood samples were tested for specific anti-*Toxoplasma* IgG using the modified agglutination test (MAT). Grey matter was dissected from each brain, using new instruments to avoid DNA cross-contamination, and DNA was extracted. Molecular diagnosis was performed using nested PCR to amplify a sequence near the 3' end of the *Toxoplasma* surface antigen 2 (SAG2) gene. PCR products from positive individuals were digested with *Hha*1 to genotype parasite strains.

**Results** The MAT titre was positive (>=1:10) in 16/100 rabbits but in 0/10 hares. This result provided serological evidence of infection in wild rabbits. DNA amplification by PCR detected the *Toxoplasma*-specific 3'SAG2 sequence in 26/100 rabbits and in 7/10 hares, providing molecular evidence of infection in both species and suggesting that 3'SAG2 PCR was a more sensitive diagnostic test than MAT. Agreement between the serological and molecular test results was incomplete (for rabbits $\chi^2$=3.119, d.f.=1, P=0.077). 19/100 rabbits and 7/10 hares were seronegative but PCR positive while 9/100 rabbits and 0/10 hares were seropositive but PCR negative. *Toxoplasma* strains were genotyped for 19 PCR positive rabbits and for four PCR positive hares, by *Hha*1digestion of 3'SAG2 PCR products, in order to distinguish Type II *Toxoplasma* from the other two clonal lineages of the parasite (Types 1 and III). Type II *Toxoplasma* has been reported to predominate in ovine infections (Owen and Trees, 1999; Dumetre *et al*, 2006). The genotyping assay revealed that 3/19 rabbit infections and 0/4 hare infections were Type II. All other infections for which the genotype was determined were Type 1 or III.

**Conclusions** The results suggest that *Toxoplasma* infection may be present in rabbits and hares without overt symptoms and without detectable specific anti-*Toxoplasma* IgG. It is not known whether such cases occur in sheep. A contrast between *Toxoplasma* strains in rabbits and hares, and strains previously reported in sheep, has been demonstrated. This result might indicate that sheep and wild herbivores do not share a common source of infection or that these host species differ in their susceptibility to different *Toxoplasma* strains.

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# Effect of ionophores monensin and lasalocid on performance and carcass characteristics of fattening Arabi lambs

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**Introduction** In some part of world for intensive production of fattening of lambs, they are fed with high amount of concentrate. However this pattern of feeding often affect negatively of rumen fermentation (Mould et al., 1983). Ionophores act by interrupting transmembrane movement and intracellular equilibrium of ions in certain classes of bacteria and protozoa that inhibit the gastrointestinal tract (McGuffey et al., 2001). In Ruminants, monensin has shown selective inhibition of ruminal bacteria, lactic and methane producers and stimulation of propionate production by 25% (Matabudul et al., 2001). In lambs, monensin improves weight gain and feed conversion ratio (FCR) and depressed the dry matter intake (DMI) (Martini et al., 1996), but lasalocid improve average daily gain (ADG) without depressed the DMI (Swanson et al., 2000). There is limited information about the effect of ionophores on performance of Iranian fattening lambs and particularly no works was done with Arabi lambs. Therefore, the present study was conducted to investigate the effects of feeding monensin and lasalocid on performance and carcass characteristics of fattening Arabi lambs.

**Materials and methods** Thirty fattening male lambs with similar conditions (22.14±0.72 Kg live weight and 90±5 day of age) from a flock of autumn lambing of Arabi sheep of the Ramin Agricultural and Natural Resources University were included in this experiment for eight weeks. Three high concentrate diets (with similar composition) containing with 30ppm monensin (M), 30ppm lasalocid (L) and none additive (Control=C) were offered to lambs in a completely random design. The diets were formulated according to NRC (1985) and were isonitrogenous (167 g/Kg DM CP) and isocaleric (12.01 MJ/KgDM ME) and had similar concentrations of other nutrients. The diets were offered ad libitum to all groups. DMI for individual lamb was estimated from difference between feed offered and refusals. The body live weight (BLW) of lambs were recorded 2 weeks interval. At the end of experiment 3 lambs for each treatment were slaughtered and physical compositions of carcass were measured. Data were subjected to analysis of variance using the GLM procedure (SAS, 2000). Initial BLW of lambs were used as a covariate for final LW, ADG and FCR.

**Results** The results of the statistical analysis of the BWG, DMI, ADG, FCR and some of the carcass characteristics are shown in table 1. ADG and BLW of fattening lambs fed the diets containing ionophores were significantly higher ($p<0.05$) than lambs fed the control diet. DMI of the lambs fed diet containing monensin were significantly lower ($p<0.05$) than other two groups. The same trend was found for FCR, while the differences was significant ($p<0.05$) only between M and C group lambs. Carcass characteristics wasn't affected by treatment ($p>0.05$), but lambs fed diet containing lasalocid had greater Dressing percentage (DP) and meat percentage (MP).

**Table 1** Effect of monensin and lasalocid on performance and carcass traits fattening Arabi lambs.

| Item | Diets | | |
|---|---|---|---|
| | C | M | L |
| BLW (Kg) | 37.53±0.789$^{b}$ | 39.10±0.885$^{a}$ | 39.27±0.647$^{a}$ |
| DMI (g) | 1190.8±30.02$^{a}$ | 1124.35±27.11$^{b}$ | 1226.78±23.25$^{a}$ |
| ADG (g) | 252.83±11.43$^{b}$ | 285.83±7.49$^{a}$ | 285.83±8.53$^{a}$ |
| FCR | 4.77±0.192$^{a}$ | 3.96±0.133$^{b}$ | 4.33±0.159$^{ab}$ |
| DP | 51.97±1.49$^{a}$ | 52.02±0.807$^{a}$ | 55.01±1.89$^{a}$ |
| MP | 50.15±3.113$^{a}$ | 50.58±2.307$^{a}$ | 52.14±2.307$^{a}$ |

(a-b): means in row with different superscripts differ significantly ($p<0.05$).

**Conclusions** These results are shown that by using both ionophore (M and L) as an additive can improve lamb growth rate. Within the experimental lamb groups, lambs fed diet (M) had lower DMI and better economic efficiency. Thus, it is recommended that, using ionophores (particularly M) as an additive would be useful for fattening lambs.

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# The effects of herbage allocation and concentrate supplementation on the performance of replacement ewe lambs offered extended grazed pastures

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**Introduction** Recent studies at this centre (Flanagan 2003, Keady et al. 2006) have shown that grazing swards in the winter (extended grazing), either in mid, late or throughout pregnancy, provides a low-cost system for wintering ewes. With decoupling of subsidy from production post Mid Term Review of the Common Agricultural Policy it is essential to improve efficiency and reduce costs of production. One of the major costs in sheep production is the cost of rearing replacements. The aim of the present study was to evaluate the effect of herbage allowance and concentrate supplementation on animal performance during extended grazing and on compensatory growth during the subsequent grazing season. Furthermore the potential herbage allowance sparing effect of concentrate supplementation was also determined.

**Materials and methods** Twelve hectares of old permanent predominantly perennial ryegrass pasture were topped to a sward height of 4.5 cm and received nitrogen fertiliser (38 kg/ha) on September 8 for grazing between December 16 and March 3. The pasture was grazed *in situ* at herbage dry matter (DM) allowances of 0.75 (L), 1.25 (M) and 1.75 (H) kg head per day as the sole diet. In addition L allowance was supplemented with 0.5 kg concentrate/head per day (LC). The concentrate consisted of barley, sugar beet pulp, citrus pulp, soyabean meal, molasses and mineral vitamins at 350, 175, 175, 250, 25 and 25 kg/t respectively. The four treatments were offered to 248 spring born ewe lambs (initial liveweight of 35.8 (s.d. 3.83) kg in a fully randomised design and was analysed using PROC GLM of SAS. *A priori* contrasts were used to test the effects of concentrate and the response of increasing herbage allowance. The ewe lambs were allocated to treatments balanced with respect to flock of origin. Pre and post grazing height and sward mass were recorded weekly for the duration of the study. The treatments, which were grazed on one replicate per treatment, were rotated weekly within the paddocks to reduce any effect of position of treatment within paddock on subsequent performance. The ewe hoggets were grazed as one flock from March 4 to August 11 to evaluate the effect of treatment on subsequent growth rate.

**Results** The swards offered in the present study had mean herbage DM mass, height and DM concentration of 2332 kg/ha, 8.15 cm and 204 g/kg respectively. The effects of grass allowance and concentrate supplementation on food intake and animal performance are presented in Table 1. Increasing grass allowance increased herbage intake, liveweight at the end of extended grazing and grazing season, liveweight gain during extended grazing and total experimental period and condition score at the end of extended grazing. Increasing herbage allowance decreased herbage utilisation during extended grazing and daily liveweight gain during the grazing season and did not alter condition score at the end of the grazing season. Concentrate supplementation increased liveweight at the end of extended grazing and grazing season, liveweight gain during extended grazing and the total experiment and condition score during extended grazing. Concentrate supplementation decreased herbage utilisation but did not alter herbage intake or condition score at the end of the study. The potential herbage sparing effect due to offering 0.5 kg concentrate/head per day during extended grazing, as determined by liveweight gain during extended grazing and the total experiment was 1.0 and 0.95 kg herbage DM respectively. Relative to treatment H, compensatory growth for treatments L and M were 0.43 and 0.0 respectively.

**Conclusions** It is concluded that increasing herbage allowance during extended grazing increased herbage intake and animal performance, and decreased herbage utilisation. Supplementing with 0.5 kg concentrate replaced 1 kg herbage DM of extended grazing herbage allowance. Compensatory growth during the grazing season did not remove the effect of extended grazing treatments on animal liveweight.

**Table 1** Effect of grass allowance and concentrate supplementation on food intake and animal performance

| | Treatment (T) | | | | | Significance | | | |
|---|---|---|---|---|---|---|---|---|---|
| Herbage allowance (kg DM/d) (HA) | 0.5 | 0.5 | 1.25 | 1.75 | | T | HA | HA | Conc v |
| Concentrate (kg/d) | 0.0 | 0.5 | 0.0 | 0.0 | s.e. | | Linear | Quad | no conc |
| Grass intake (kg DM/d) | 0.61 | 0.57 | 0.92 | 1.24 | 0.06 | *** | *** | NS | NS |
| Grass utilisation (g/kg) | 822 | 727 | 733 | 684 | 30.7 | * | ** | NS | * |
| Liveweight (kg) at end of: | | | | | | | | | |
| extended grazing | 35.8 | 42.2 | 39.8 | 42.3 | 0.32 | *** | *** | * | *** |
| grazing season | 52.5 | 55.8 | 53.4 | 56.2 | 0.64 | *** | *** | NS | *** |
| Liveweight change (g/d) during: | | | | | | | | | |
| extended grazing | -1.0 | 84 | 52 | 84 | 4.2 | *** | *** | P=0.06 | *** |
| grazing season | 103 | 83 | 85 | 88 | 4.0 | ** | *** | * | *** |
| total study | 69 | 83 | 73 | 85 | 2.7 | *** | *** | NS | *** |
| Condition score at end of : | | | | | | | | | |
| extended grazing | 2.77 | 3.18 | 2.91 | 3.03 | 0.034 | *** | *** | NS | *** |
| grazing season | 3.17 | 3.25 | 3.16 | 3.30 | 0.055 | NS | NS | NS | NS |

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# Assessment of dairy sheep internal fat depots by real time ultrasound and image analysis

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**Introduction** In sheep production the ability of the animal to retain and mobilize body fat reserves is of considerable importance in determining the sheep productivity or even its survival. The most common way to predict body fat reserves is the body condition score. On the other hand, it is accepted that breeds have a different fat distribution within the body (Taylor *et al.*, 1989). In general, ewes breed for milk production tend to deposit more fat in internal depots and those breed for meat production deposit more fat in the carcass depot (Frutos *et al*., 1997). This kind of fat distribution can explain the use of an objective and more precise way to predict the internal fat in ewes of the Churra breed, in which the internal fat depots plays an important role. Thus, it is reasonable hypothesize that the internal fat depots are related with muscle and subcutaneous fat measurements. As a result, the study herein reported was undertaken to achieve the relationship between real time ultrasound measurements obtain over thoracic, lumbar and sternum regions to predict the internal fat depots of Churra da Terra Quente (CTQ) milk breed.

**Materials and methods** Forty-seven female sheep of the native CTQ breed (41.9±6.8 kg) were used in this study. Just before slaughter, the animals were scanned with an Aloka real time scanner using one linear probe of 7.5 MHz. Sheep were individually restrained in a crate to minimize movements and ensure they were standing in a similar stance. The probe was placed perpendicular to the backbone, over the 13$^{th}$ thoracic vertebra, between the 3$^{rd}$ and the 4$^{th}$ lumbar vertebrae and over the 3$^{rd}$ sternebra of the sternum. The measurements of subcutaneous fat depth at these sites represented three fat depths - SC13, SC34 and SCst, respectively. The depth of the *Longissimus thoracis et lumborum* muscle was measured over the 13$^{th}$ thoracic vertebra and at the interval between the 3$^{rd}$ and 4$^{th}$ lumbar vertebrae yielding two muscle depths - MD13 and MD34, respectively. The images were analysed with Image J software. After slaughter, the internal fat depots (mesenteric, omental and kidney and pelvic) were carefully obtained and weighed. The relationships between fat depots and ultrasound measurements were estimated by single regression equations. All regression analyses were performed with SAS software. The regression equations were evaluated by the coefficient of determination ($r^2$) and residual standard deviation (rsd).

**Results** In Table 1 are presented $r^2$ and rsd of the simple linear regression equations between *in vivo* ultrasound measurements and the amount and proportion of fat depots.

**Table 1** Coefficients of determination ($r^2$) and residual standard deviations (rsd) for simple linear regression equations between *in vivo* ultrasound measurements (independent variable) and the amount (g) and proportion (g kg$^{-1}$ BW) of fat depots (dependent variable).

| Fat depot | | *In vivo* ultrasound measurements | | | | |
|---|---|---|---|---|---|---|
| | | SC13 | SC34 | MD13 | MD34 | SCst |
| Omental fat, kg | $r^2$ | 0.781*** | 0.788*** | 0.653*** | 0.676*** | 0.023$^{ns}$ |
| | rsd | 0.47 | 0.47 | 0.55 | 0.53 | 0.90 |
| Mesenteric fat, kg | $r^2$ | 0.717*** | 0.773*** | 0.427*** | 0.545*** | 0.025$^{ns}$ |
| | rsd | 0.19 | 0.18 | 0.24 | 0.22 | 0.32 |
| Kidney and pelvic fat, kg | $r^2$ | 0.724*** | 0.742*** | 0.523*** | 0.549*** | 0.010$^{ns}$ |
| | rsd | 0.38 | 0.37 | 0.47 | 0.46 | 0.68 |
| Omental fat, g kg$^{-1}$ BW | $r^2$ | 0.488*** | 0.489*** | 0.596*** | 0.407*** | 0.125* |
| | rsd | 11.3 | 11.3 | 10.4 | 12.1 | 14.8 |
| Mesenteric fat, g kg$^{-1}$ BW | $r^2$ | 0.655*** | 0.687*** | 0.533*** | 0.431*** | 0.315** |
| | rsd | 3.8 | 3.7 | 4.2 | 4.5 | 4.9 |
| Kidney and pelvic fat, g kg$^{-1}$ BW | $r^2$ | 0.677*** | 0.681*** | 0.432*** | 0.445*** | 0.011$^{ns}$ |
| | rsd | 7.2 | 7.2 | 9.0 | 8.9 | 11.8 |

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; $^{ns}$ not significant $P > 0.05$.

The results show that the thoracic and lumbar measurements (SC13, SC34, MD13 and MD34) can explain a large amount of the variation of the dependent variables ($r^2$ ranged from 0.41 to 0.79). This was particularly evident with the SC13 and SC34 measurements as predictors of the amount of internal fat depots ($r^2$ ranged from 0.72 to 0.79). However, the potential of SCst ultrasound measurements ($r^2$ ranged from 0.01 to 0.32) was clearly below the other measurements.

**Conclusions** *In vivo* ultrasound thoracic and lumbar measurements are able to estimate internal fat depots. Therefore, this technique show potential to monitoring changes on ewe fat reserves. Generally the fat depots are overestimated and underestimated by ultrasound fat and muscle measurements, respectively.

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# The effect of an oral drench trace-element supplementation programme on lamb production in a commercial sheep flock in England

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**Introduction** In the UK lamb mortality is a major factor limiting profitability of sheep operations ranging from 15-22% from birth to weaning with the majority occurring in the first 24h of life (Teagasc 1991). Lamb survival to some extent is affected by micronutrient status of the ewe pre-lambing, which can be manipulated by dietary supplementation with subsequent effects on vitality and resistance to infections in the lamb (Rook *et al*. 2004) inadequate dietary supply of micronutrients to the lamb at later growth stages, will affect resistance to nematode and bacterial infections (Suttle and Jones 1989) and hence efficiency of growth to slaughter weight – the second major factor for profitability. The practicalities of management, labour time and concerns of cost-effectiveness, often result in no micronutrient supplementation of the sheep or the use of a drench at critical times in the sheep production cycle as a quick-fix to potential micronutrient deficiencies in sheep. This study was carried out to evaluate a micronutrient drench supplementation programme as a means of improving productivity and profitability of lamb production under commercial conditions.

**Materials and methods** 120 twin bearing mule ewes (Masham x Swaledale) mated with Texel and Suffolk rams were split into two groups, matched for body condition score (2.9± 0.05 SEM), at 4 weeks prior to predicted lambing date. All ewes were fed a diet based on silage and a commercial ewe concentrate feed and kept indoors. Silage was analysed for major mineral and trace-element composition. The ewes in the trial group received a single 20ml oral dose with a commercial micronutrient drench fortified with chelated trace-elements at the beginning of the trial. At parturition date of birth, the number of lambs born and birth weight were recorded and lambs tagged according to treatment group of the ewes. Ewes and their lambs were taken out into fields within 48 hours post-lambing. Any ewe and lamb losses were recorded. At 6 weeks of age, all lambs were wormed with an oral drench. The lambs in the trial group received a single 7.5ml oral dose of a commercial micronutrient drench fortified with chelated trace-elements at worming and at weaning. Lambs were weighed at worming, weaning and slaughter. Data were analysed by one-way analysis of variance using dietary treatment as a factor (Genstat Version 9). Lamb birth weights were used as covariate in analysis of growth rates.

**Results** There was no significant difference between treatments in ewe body condition score at weaning (Mean control= 2.3 v drench 2.2, SED 0.133, P=0.490). A total of 6 ewes were lost prior to lambing for abortion or other health reasons. The silage fed to the ewes was deficient in most major minerals and trace-elements and contained increased levels of Iron and Aluminium. Birth weights and number of lambs born alive (mean 1.97 ±0.017 SEM ) were similar between treatments. The majority of lambs born were Suffolk cross-bred (195/225). Texel cross-bred lambs born to ewes were evenly distributed between treatments. Lamb growth rates from birth to worming (40 days) were not significantly affected by supplementation of ewes pre-lambing. Subsequent growth rates until weaning did not differ significantly between treatment groups.

**Table 1** Effects of micronutrient supplementation using an oral drench feeding regime pre-and post lambing on lamb growth to weaning

| | Control | Micronutrient drench programme | P-Value | SED |
|---|---|---|---|---|
| Total no. of lambs born alive | 114 | 111 | - | - |
| Birth weight (kg) | 5.34 | 5.37 | 0.842 | 0.152 |
| Age at weight 2 (days) | 40 | 39 | 0.173 | 0.846 |
| Weight 2 (kg) | 16.90 | 16.99 | 0.809 | 0.370 |
| Age at weaning (days) | 112 | 111 | 0.173 | 0.846 |
| Weaning weight (kg) | 32.27 | 31.75 | 0.349 | 0.551 |
| Overall Growth rate (g/d) | 240 | 238 | 0.598 | 4.46 |

**Conclusions** The data does not suggest a benefit from a micronutrient supplementation programme with a commercial micronutrient drench at critical stages of the sheep production cycle, for growth until weaning. The commercial concentrate fed to ewes contained some trace-elements and vitamins. Total daily micronutrient intakes in ewes prior to lambing are going to have be taken into account. The consequences for lamb survival, growth to slaughter and carcass characteristics are currently being evaluated.

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# Genetic variability in beta-lactoglobulin, calpastain and calpain loci in Kurdi sheep

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**Introduction** DNA-based molecular methods have made possible genotyping of animals of any age and sex for milk and meat genes, thus providing a potentially more efficient and flexible selection tool. Among specific genes that may affect economically important traits in sheep, the Beta-lactoglobulin (BLG) locus has been extensively studied [Barillet et al., 2005]. The genotype BB of BLG seems to be associated with higher milk yield; on the other hand genotypes AA and AB seem to be superior in protein and casein content and crude yield [Garzon et al., 1992]. Another genes intensively investigated in farm animal are that of calpastatin (CAST) and calpain (CAPN). Calpastatin and calpain (CAPN) deserves special attention because of their major role in meat production. The calpain-calpastatin system (CCS) comprises a family of calcium dependent neutral proteases. The CAPNs have been shown to play the major role in post mortem tenderization in beef, lamb, and pork by degrading specific muscle structural proteins. The aim of the present study was to identify genotypes of BLG, CAST and CAPN genes in Kurdi sheep breed by PCR.

**Materials and methods** Blood samples were randomly collected from 100 pure bred Kurdi sheep from Kurdi breeding station located in Shirvan, Mashhad, Iran. DNA was extracted from 100 µl of blood by Guanidinium-Thiucianate Silica gel. Method. 1 µl DNA was amplified in a total volume of 20 µl PCR mix using the Biometra T Personal Ver: 1.11 thermocycler. Primers were designed by Primer Premier 5 software according to X12817 Gene Bank accession number for Beta-lactoglobulin, AY834765 for CAST and J05065 for CAPN. Products of amplification were recognized by electrophoresis on 1.5% agarose gel stained with etithium bromide. Five µl of PCR product was incubated for 5 h at 37 °C with 5 units of *Rsa*I and *Msp*I enzymes for BLG and CAST genes, respectively. Digestion products of were separated by electrophoresis on 8% non-denaturant polyacrylamid gel and visualized after silver staining. Polymorphisms of CAPN locus were detected using SSCP method on 8% non-denaturing polyacrylamid gel with 10 % glycerol. The frequencies of genotypes, alleles, mean expected, observed and Nei's heterozygosities and Hardy-Weinberg equilibrium test were calculated using PopGene32 (ver 1.31) program [http://cc.oulu.fi/~jaspi/popgen/popdown.htm].

**Results** Three loci were polymorphic in Iranian Kurdi sheep. The genotypes AA/AB/BB for the BLG, MM/MN for the CAST and AA/AB for the CAPN locus were observed. Table 1 shows the allele frequencies for BLG, CAST and CAPN genes in the Iranian Kurdi sheep

**Table 1:** Allelic and genotype frequencies, observed heterozigosity, expected heterozigosity, average heterozigosity and Nei values for BLG, CAPN and CAST loci.

| Locus | A | B | AA | AB | BB | Obs_Het | Exp_Het* | Nei** | Ave_Het | $\chi^2$ |
|---|---|---|---|---|---|---|---|---|---|---|
| BLG | 0.51 | 0.49 | 24% | 54% | 22% | 0.5400 | 0.5023 | 0.4998 | 0.4998 | 0.5686 |
| CAPN | 0.96 | 0.04 | 92% | 8% | 0% | 0.0824 | 0.0794 | 0.0790 | 0.0790 | 0.1336 |
| CAST*** | 0.88 | 0.12 | 76% | 24% | 0% | 0.2400 | 0.2123 | 0.2112 | 0.2112 | 1.7743 |

* Expected heterozygosity were computed using Levene (1949)
** Nei's (1979) expected heterozygosity
***Alleles for CAST locus have shown in text with M and N but in table M=A and N=B.

**Conclusions** Our results showed that PCR-RFLP and PCR-SSCP were appropriate tools for evaluating genetic variability. This study was the first using polymorphism of BLG, CAST and CAPN loci to understand genetic variability of Kurdi sheep in Iran. The present study may be regarded as the beginning of attempts to understand the genetic variability of native sheep breed in Khorasan state and identification of association between genotypic variants and productive parameters for future study.

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# The effects of steam treated sugarcane pith as a feed ingredient on performance of lactating dairy goats

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**Introduction** Sugarcane bagasse and pith, by-product which the residue after rind removal, are highly lignified by products of the sugar and paper industries, are the most abundant by-product in Iran. The use of by-product in animal nutrition is necessity since it may increase the availability of feed for animal and avoid accumulation that contributes to environmental problems. The main nutritional constraints for these crop residues as animal feeds are their slow rate of digestion and low nitrogen content (Liu et al., 2000). Steam-pressure treatment cleavage the bounds between lignin and the other component of the cell wall, in order to improve it's degradability by enzymes of the rumen microbial ecosystem. The aim of this study were to evaluate the effect of increasing dietary steam treated pith content on feed intake, milk yield and composition of lactating dairy saanen goats.

**Materials and methods** The steam treated pith (STP), was prepared at 20 bar for 3 min. Eight lactating saanen goats were randomly assigned to a replicated 4*4 Latin square design (with 2 different squares for balancing carryover effects), with 4 treatments and four periods of 21d each (adaptation, 14d; sample collection, 7d ). Diets contained 48% concentrate, 30% lucerne hay, and 12% wheat straw and pith (dry matter basis). The 4 treatments were substitution of 0, 4, 8 and 12% STP by wheat straw in rations. Diets were fed for ad libitum intake twice daily at 900 and 2100 h. The amount of feed offered was adjusted daily to obtain approximately 10% refusals (as-fed basis). During the sample collection period DMI was measured daily for all goats. Feed, feces and refusals samples were collected during the last 7 d of each period and composited by animal within period, dried at 60°C, ground (1-mm screen) and analysed for nutrients. Goats were milked twice daily. Milk production was recorded, and milk was sampled at each milking during the last 7d of each period. Milk samples were analysed for protein, fat and lactose (AOAC, 2002). Data were analysed using the GLM procedure (SAS, 2002).

**Results** Steam treated pith has non-significant effect on intake of dry matter (P>0.05). Numerically feed DMI was greatest for goats fed the 8% steam treated pith diet (table 1). Yields of milk increased quadratically with increasing dietary steam treated pith (P<0.05), but FCM did not significantly affect by level of STP in diet (P>0.05). Milk yield for the 8% steam treated pith diet was greater than for controls and 12%. Yields of FCM increased numerically with greater steam treated pith content of the diet, except four treatments. Milk fat percentage decreased linearly with addition of steam treated pith to the diet (P<0.05). The 8% steam treated pith diet has greatest milk protein and SNF percentage (P<0.05). There were not significant differences in digestibility of DM and NDF between treatments. But with increased STP content of diet, digestibility was greater (P>0.05).

**Table 1** Effect of steam treated pith on DMI, milk yield, milk composition, and digestion of DM and NDF

| | Steam treated sugarcane pith in total diet† | | | | | |
|---|---|---|---|---|---|---|
| Item | 0% | 4% | 8% | 12% | SEM | effect |
| DMI, kg/d | 2.28 | 2.32 | 2.34 | 2.32 | 0.023 | NS |
| Milk yield, kg/d | 1.79 | 1.881 | 1.94 | 1.81 | 0.032 | *Q |
| Milk fat, % | 3.51 | 3.42[b] | 3.30 | 3.13[c] | 0.049 | *L |
| FCM, kg/d | 1.66 | 1.71 | 1.72 | 1.61 | 0.062 | NS |
| Milk protein, % | 2.84 | 2.82 | 2.92[b] | 2.85 | 0.016 | C* |
| Milk lactose, % | 4.26 | 4.24 | 4.21 | 4.27 | 0.099 | NS |
| SNF, % | 7.79[a] | 7.70[a] | 7.97 | 7.76 | 0.041 | *L |
| Digestibility | | | | | | |
| DM, % | 59.1 | 63.4 | 64.3 | 63 | 1.33 | NS |
| NDF, % | 42.5 | 49.7 | 51.4 | 48.1 | 2.24 | NS |

†. Dry basis  Q= Quadratic effect  L= Linear effect  C= Cubic effect

**Conclusions** The results of the present study indicated that use the STP caused to significant increased in milk yield and composition, and numerically increased digestibility of dry matter and neutral detergent fibre. Therefore steam-pressure treatment improved nutritional value of sugarcane by-product for ruminants animal and had more nutritional characteristic compare with wheat straw.

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# Effect of organic zinc and selenium on dairy cow productivity and fertility.

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**Introduction:** Major changes have been occurring over the last 20 years in the UK dairy industry, with improvements in cow genetics and nutritional management resulting in a doubling of average milk yields to 6,750 litres/cow/year by 2004 (DEFRA, 2005). The same period has resulted in a decline of dairy cow fertility parameters with recent studies having identified a 1% fall in conception rates per annum (Royal *et al*., 2000). The role of organic forms of trace minerals has been examined in ruminant diets with particular regard to their role in immune function (Spears, 2000). The aim of the study was to examine the effect of feeding an organic source of zinc (Bioplex Zinc™, Alltech Inc. USA) and selenium yeast (Sel-Plex™, Alltech Inc. USA) to high yielding Holstein dairy cows on production and reproductive performance.

**Materials and Methods**: 88 Holstein cows were assigned to one of two treatments at calving on the basis of parity, previous yield and calving interval. Cows calved between September 2004 and April 2005. Cows were fed a semi-TMR mixture of grass silage, whole crop wheat silage, wet brewers grains, molasses and 7.5kg of treatment concentrates (for maintenance plus 28 litres) with standard concentrates fed to yield in the parlour at a rate of 0.4kg per litre of milk above 28 litres. The semi-TMR was fed to provide 105% of appetite. The treatment concentrate provided an additional 6mg/day Se, as Sel-Plex™, and 300mg/day Zn, as Bioplex Zn™. Each cow completed a 305-day lactation. Milk yield was recorded at each milking with samples being taken once per month at pm and am milking for fat, protein and somatic cell count analysis (CIS, Paisley, UK). Group feed intakes of the semi-TMR were measured every two weeks, with body condition scoring carried out monthly (starting 1 month prior to calving). Fertility was recorded using Interherd (NMR) with the end of the service period being on the 8th August 2005. Statistical analysis was carried out using one-way ANOVA*, Kruskal-Wallis one-way Anova** and Chi-squared test*** as appropriate. A 95% confidence interval was used.

**Results**: There was no effect (P>0.05) of treatment on milk yield, milk fat, milk protein or milk somatic cell count (SCC). There was also no effect on dry matter intake or any incidence of disease (P > 0.05). Table 1 shows that there was no effect of treatment on the calving to 1st service interval, services per conception or conception rate to 1st service. Cows fed the Bioplex Zinc™ and Sel-Plex™ had a tendency (P = 0.09) towards a higher conception rate over all services (31.1% vs. 21.2%), and lower (P = 0.08) 200 day empty rate (27.5% vs. 45.2%). However, none of these differences were statistically significant (P>0.05). There was a (P<0.05) reduction in the number of empty cows at the end of the service period (17.5% vs. 40.5%) when fed the Bioplex Zinc™ and Sel-Plex™.

**Table 1** Effect of Bioplex Zinc™ and Sel-Plex™ addition on fertility in dairy cows.

| | | Control | Treatment | Significance |
|---|---|---|---|---|
| Calving to 1st visible oestrus* | (days) | 65.4 | 65.9 | ns |
| Calving to 1st Service* | (days) | 73.8 | 78.9 | ns |
| Services per conception** | (n) | 2.5 | 2.4 | ns |
| Conception rate to 1st service*** | (%) | 14.3 | 25.0 | ns |
| Conception rate over all services*** | (%) | 21.2 | 31.1 | 0.09 |
| 100 day pregnancy rate*** | (%) | 21.4 | 35.0 | ns |
| Empty rate at end of service period*** | (%) | 40.5 | 17.5 | <0.05 |
| 200 day empty rate*** | (%) | 45.2 | 27.5 | 0.081 |
| Calving to Conception** | (days) | 118 | 119 | ns |

**Conclusions** The reproductive performance of the control group was particularly disappointing in this study, with improvements observed in the treatment group still being below UK national average. Feeding Bioplex Zinc™ and Sel-Plex™ to dairy cows reduced the number of cows not in calf at the end of the service period. Although not significantly different, there were also strong trends for improvements in overall conception rate and 200-day not-in-calf rate.

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# Relationship between type characteristics and length of productive life in Iranian Holstein dairy cattle

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**Introduction** Type traits have been used as indirect measures of selection for herd life in last few decades, because type traits can be measured earlier in first lactation, they have relative higher heritability than longevity traits and are correlated moderately with herd life. Increased herd life leads to lower replacement costs, longer use of high performance animals in their herds and also obtaining more progenies from high yielding animals.

**Materials and methods** Type traits data of 7225 first lactating animals were used in order to estimate genetic and phenotypic correlation between type and longevity traits. This information was obtained from Animal Breeding Centre of Iran, containing 18 linear type traits and other data such as pedigree information and milk production. Type traits had been measured on basis of 1-9 scoring and also by direct measuring. Heritability, genetic and phenotypic correlations were estimated using single and multiple trait animal models with DF-REML method. Data of type traits were adjusted for fixed effects using the method of Foster (1989). True length of productive life was adjusted for age at first calving using the same method of Dentine (1987) and Boldman (1992) which achieved productive herd life data. We used the method of Ducrocq (1986) to calculate the functional length of productive life (FLPL) of animals.

**Results** Longevity traits had almost low heritability and were found to be 0.02993 and 0.04139 for true and functional productive life traits respectively. Type traits had moderate heritability ranging from 0.0622 for angularity to 0.431 for body depth. Genetic and phenotypic correlation had moderate values but genetic correlations were almost higher than phenotypic correlations. Most of udder traits had positive high correlations with productive herd life, where the highest values belonged to udder depth, fore udder attachment and rear udder width correlated with herd life. Body traits had negative genetic and phenotypic correlations with herd life where the highest negative values belonged to stature, body depth and size correlated with herd life.

**Table 1** Genetic and phenotypic correlations of true length of productive life(TLPL), functional length of productive life(FLPL) with 18 type traits.

| Type Traits | TLPL | | FLPL | |
|---|---|---|---|---|
| | Genetic | Phenotypic | Genetic | Phenotypic |
| RUW | 0.3701 | 0.2046 | 0.4836 | 0.1929 |
| RUH | 0.3073 | 0.2031 | 0.2987 | 0.1888 |
| FUA | 0.3230 | 0.1470 | 0.4734 | 0.1308 |
| UDD | 0.5541 | 0.2716 | 0.5755 | 0.0410 |
| FTP | 0.3015 | -0.0128 | 0.4627 | 0.0286 |
| RTP | 0.1570 | -0.0427 | 0.3039 | -0.0028 |
| FTL | 0.1529 | 0.1120 | 0.1600 | 0.0830 |
| ANG | 0.5070 | 0.5570 | 0.5335 | 0.1115 |
| BOD | -0.3340 | -0.2540 | -0.4197 | -0.2465 |
| CHW | 0.1550 | 0.0800 | 0.1432 | 0.0846 |
| FAN | -0.1984 | -0.0246 | -0.1548 | -0.0323 |
| PNW | -0.3237 | -0.273 | -0.3423 | -0.2872 |
| RLS | 0.2356 | 0.1452 | 0.1527 | 0.1142 |
| SLG | 0.3310 | 0.1650 | 0.3134 | 0.1465 |
| RUL | 0.4702 | 0.2713 | 0.3377 | 0.2668 |
| SIZ | -0.3808 | -0.2739 | -0.3720 | -0.2761 |
| STA | -0.3896 | -0.2744 | -0.3811 | -0.2791 |

**Conclusion** Low heritability and also time demanding measurement of longevity traits lead to select animals for these traits using correlated traits with higher heritability. Type traits with moderate to high heritability have good transmitting abilities from generation to generation. The use of correlated selection can result in improved longevity traits and postpone the involuntary culling. Results showed that animals with optimal udder characteristics and smaller bodies tend to live longer than others.

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# The effect of palm oil fatty acids on cervix regression in dairy cows during the first 40 days postpartum

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**Introduction** An undisturbed re-organisation of the cervix postpartum is a basic pre-condition for an undisturbed puerperium (Wehrend *et al*., 2003). This aspect of the puerperal involution has been insufficiently considered in the literature. It was shown that some cows developed a disorder of the cervical involution (Wehrend and Bostedt, 2003). LeBlank *et al*. (2002) concluded that the size of cervix in cows with clinical endometritis were associated with a decrease in pregnancy rate. With the use of the ultrasonic technique instead of using fingers of the human hand, more exact data on cervix regression can be delivered. Meanwhile, little is known about the effect of diet on cervix regression after parturition. The objective of this study was to evaluate the effect of palm oil fatty acids on cervix regression during the first 40 days postpartum in Iranian Holstein dairy cows using ultrasonic technique.

**Materials and methods** From d 5 to 40 postpartum (PP), sixteen cows were fed isonitrogenous diets containing 0 (n=8) or 2.5% (n=8) palm oil fatty acids (POFA; Bergafat; Berg + Schmidt GmbH, Hamburg, Germany). The ultrasound instrument (Medison SA 600V, Seoul, Korea), equipped with a linear-array, 7.5-*MHz* rectal transducer, was used to monitor cervical diameter at 3-d intervals from 10 to 40 d postpartum. Cervical diameter was defined as the distance from the dorsal to the ventral surface of the cervix. The data were analyzed using the MIXED procedure of SAS (2001) for a completely randomised design with repeated measures. The model contained the effects of treatment, time, cow within treatment and the interaction of treatment by time. The overall effect of treatment was tested using cow within treatment as the error term. Least squares means are reported throughout, and significance was declared at $P< 0.05$.

**Results** The pattern of cervix regression is shown in Figure 1. The cervix regression was similar among diets ($P= 0.20$) but tended to regress more rapidly in the supplemented group. The cervix diameter mean was 37.03 and 35.78 mm (SEM= 0.7) for control and the supplemented diets, respectively. The effect of time was significant ($P< 0.001$) and the cervix diameter was reduced over the time but the interaction of diet and time was not significant.

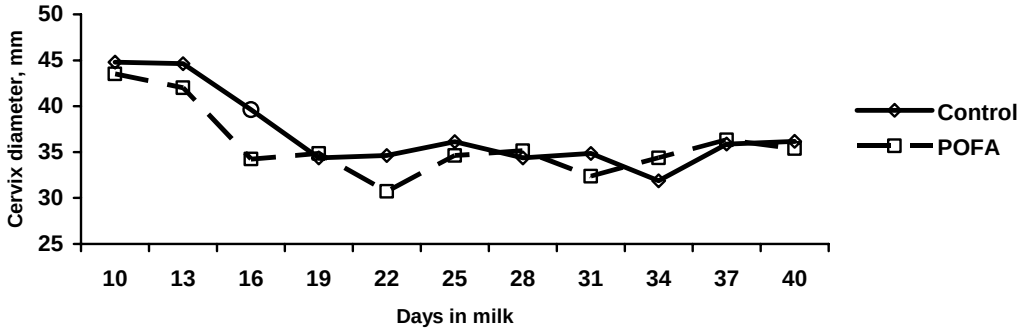


**Figure 1** Cervix regression in early lactation cows fed diets containing 0 (control) and 2.5% palm oil fatty acids (POFA) during the first 40 days postpartum.

**Conclusions** The cervical involution process was not constant and parts with different reduction rates were distinguished. It was remarkable that the cervical opening occurs chronologically in parallel with the intrauterine, puerperal modification and degradation processes, particularly in the area of the caruncles (Wehrend *et al*., 2003). The results of the present study also demonstrated that dietary supplementation with palm oil fatty acids in early lactation dairy cows had no apparent effect on cervical regression.

**Acknowledgements** Financial support from Excellence Centre for Animal Science of Ferdowsi University of Mashhad is gratefully acknowledged.

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# Effect of first 60-day cumulative milk yield on days open in Holstein dairy cows in Iran

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**Introduction** Reproduction and milk production are the principal factors that are influencing dairy farm profitability. The dairy industry in Iran has changed dramatically in the last decade. The shift toward more productive cows and larger herds in Iran is associated with a decrease reproductive efficiency (Heravi Moussavi et al., 2004). Increased knowledge about the principal causes of reduced fertility is essential. The root cause of the declining fertility is probably a combination of a variety of physiological and management factors that have an additive effect on reproductive efficiency. Dairy cattle are inseminated and pregnancy is established while dairy cows are lactating. Based on the analyses of large datasets, there is clearly an antagonistic relationship between milk production and reproduction in dairy cattle (Lucy, 2001). It was shown that the hazard ratio for cumulative first 60-day milk yield and conception in high producer cows was 8 percent less than the others and also high milk yield was a risk factor for several reproductive disorders (Grohn and Rajala-Schultz, 2000). The objective of this study was to evaluate the effect of first 60-day cumulative milk yield on days open in Iranian Holstein dairy cows.

**Materials and Methods** A data set of 14,000 lactations during 1996 to 2004 from six large dairy herds were used for this study. The first 60-day cumulative milk yield in the corresponding lactation period in which cow got pregnant was recorded. Individual cumulative 60-day milk yield was divided into five categories and these categories, rather than the continuous variable from which they were derived, were used for studying the effect of milk yield on days open. The days open was defined as the interval from 35 d after calving to either conception or 450 d after calving, whichever came first. The data were analyzed using the GLM procedure of SAS (2001) for a completely randomized design. Significance was declared at $P < 0.05$. Means were separated by Duncan multiple range test.

**Results** Figure 1 shows the trend in the 60-day cumulative milk yield in Iranian Holstein cows during 1996 until 2004. Year had significant effect on the milk yield ($P < 0.001$). Except than 2002, which was a drought year, the cumulative milk yield increased linearly during the years. The effect of the 60-day cumulative milk yield on days open is shown in Figure 2. Days open was impacted by the milk yield ($P < 0.01$) and cows yielding milk more than 2196 kg during the first 60-day postpartum had greater days open compare with the lower producers.

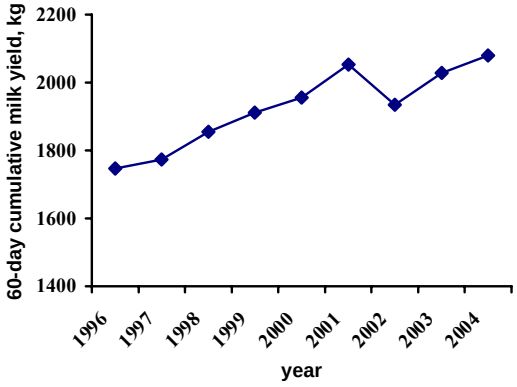


**Figure 1** The 60-day cumulative milk yield during 1996 until 2004 in Iranian Holstein cows

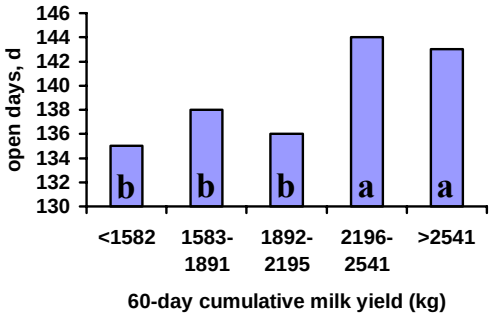


**Figure 2** Effect of 60-day cumulative milk yield on days open in Iranian Holstein cows (r= 0.03)

**Conclusions** The results demonstrate an increase in 60-day cumulative milk yield during the study period. The results also show greater days open in the high producer cows compare with the lower ones. More study is needed to elucidate other factors contributed to the reduced reproductive performance in dairy cows.

**Acknowledgements** Financial support from Ferdowsi University of Mashhad is gratefully acknowledged.

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# Seasonal changes in milk yield and fat content in Holstein dairy herds of Khorasan province

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**Introduction** Variation in milk production is depended on different factors including: temperature, humidity and day length, which are changing at different months of year (Saremi et al., 2003, Naerian et al., 2003). Dairy industry sustain loss or make profit according to amount of milk production or ingredients during year (Barbano et al., 1991). Traditional Bazaar and dairy industry are influenced by milk processing technology so that increased shelf time of products and whole milk and its products having the opportunity to distribute more effective (Erba et al., 1996). Seasonal changes in milk production leads to more processing of milk and changes in total solids of milk , which can influence price of milk (Stephenson et al., 1989). This study designed to investigate seasonal changes in milk production and its fat content in addition to investigating these changes between years (1988-2003).

**Materials and methods** All governmental records of Holstein herds in Khorasan Razavi state, Northern Khorasan state and Southern Khorasan state between years 1988 to 2003 were collected from Jihad-e Agriculture Organization of Khorasan, Animal Science Centre. Collected records were 65536 included: milk production (Kg), milk fat (%) and recording date. Records were obtained from herds that were under recording system just like American DHI. Data were analyzed in a completely randomized design using SAS 6.12. Means were compared using Duncan test ($P<0.05$).

**Results** As can be seen in figure 1 seasonal changes in milk yield and its ingredients take place at Khorasan state extensively so lead to fluctuations in milk supply. Figure 1 shows that peak milk production takes place at summer specially July and August ($P<0.01$) with low fat content ($P<0.01$). Adversely at autumn and winter there is least milk production ($P<0.01$) with a minimum at November and December, whereas there is most demand for milk at this time. Trends of milk production and fat % between years 1988 up to 2003 had been shown at figure 2. There is a huge increase in milk yield and fat % in 1988 to 1993 ($P<0.01$). After 1993 there is a bit reduction and increase in milk yield and composition.

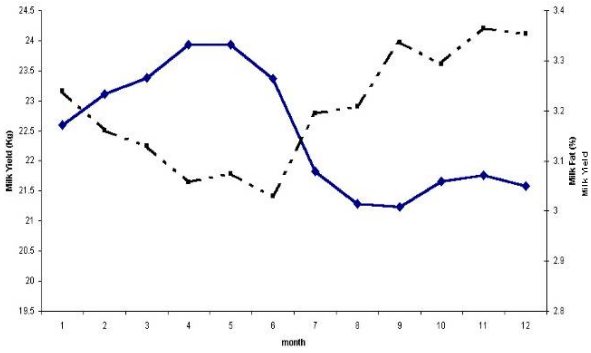


**Figure 1** Effect of month of lactation on milk production and its fat %

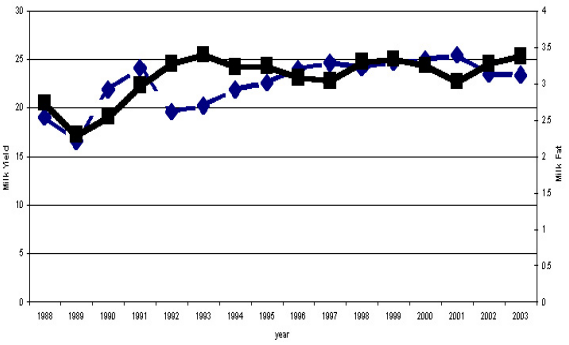


**Figure 2** Effect of year on milk production and its fat%

**Conclusions** At summer there is peak temperature at most parts of Iran especially Khorasan state, which can have negative effects on milk production and composition so that cows can't show their genetic potential. In addition, at this time in order to high temperature there is least demand for milk and dairy industry can't buy milk from farmers. Low fat content leads to higher processing. Low milk products derive from autumn and winter milk. There is most milk demand during these seasons. It seems that planning for reducing these fluctuations in milk yield production at Khorasan states is necessary and could make a balance between supply and demands in milk marketing.

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# Reasons and timing of cows leaving herd in dairy cows using survival analysis methodology

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**Introduction** Cow longevity is highly related to dairy farm profit. Cows are culled for a variety of reasons. The predominant reasons for culling are reproduction (i.e., failure to conceive), health, and low production (Bascom and Young, 1998). Half of the herd removals occur involuntarily and prematurely because of health disorders (Beaudeau *et al*., 2000). The decision to cull is a complex one. Farmers may consider many individual (such as age, stage of lactation, milk production, health status, and reproductive performance) and economic (such as milk price, the price of culled cows, and the price and availability of replacement heifers) factors when deciding to cull a cow. On the other hand, the risk of culling is not consistent across all stages of lactation. Cows experience the highest risk shortly after calving (Fetrow *et al*., 2006). Survival analysis allows for a more appropriate management of censored data and time-dependent covariates. Analyses of the reason and timing of culling is needed to predict herd performance. The objective of this study was to study the reasons and timing of cows leaving herd in two large Holstein dairy farms in Iran.

**Materials and methods** The culling data from two large dairy farms during 1999 until 2004 were used. All cows in the study were Holstein. Milking occurred 2 or 3 times daily in milking parlour. During the period, the median number of cows in the study herds was 800. Data on cow and herd identification, calving and culling reasons and dates were recorded. The farmers had a list of reasons why a cow leaving herd including death but the main culling reasons except than death were low milk production, feet and legs problems, reproductive problems, mastitis, diseases, and udder problems. The dependent variable of interest was the number of days between calving and culling. The data on the days in milk (DIM) when a cow was culled for the defined categories were analyzed using the GLM procedure of SAS (2001). Number of days between calving and culling was analyzed by survival analysis. Survival statistical analysis was performed using the statistical software package JMP (SAS Institute Inc., NC, USA). The days between calving and culling was defined for an uncensored cow as the interval from calving to 305 d after that and for a censored cow as the interval for more than 305.

**Results** The culling probabilities were 0.03, 0.04, 0.4, 0.01, 0.15, 0.29, and 0.08, respectively for low milk production, feet and legs problems, reproductive problems, death, mastitis, diseases, and udder problems. The DIM when a cow was culled was affected by the culling reasons ($P < 0.001$) and it was greater for the low milk production and reproduction problems (Fig 1). Figure 2 shows the survival analysis curve for the days between calving and culling. The median time was 228 d.

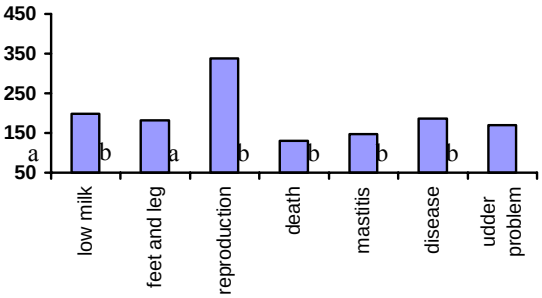


**Figure 1** Days in milk when a cow was culled based on the culling reasons

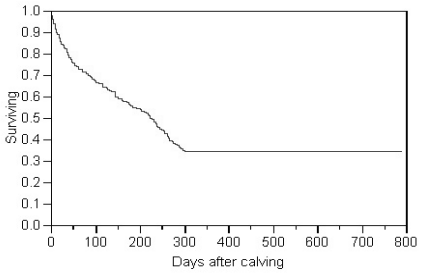


**Figure 2** Survival analysis curve for the culled cows

**Conclusions** The results of the present study demonstrate that reproduction problems were the main reason to cull cows during the study period followed by diseases. The median time when a cow was culled suggested that it was preferred to cull a cow far after milk peak instead of culling shortly after calving. This research also indicates that dairy farmers consider many factors including disease, milk yield, conception status and stage of lactation, when deciding whether and when to cull a cow.

**Acknowledgements** Financial support from Ferdowsi University of Mashhad is gratefully acknowledged.

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# Effect of level of fish oil in the diet on flow of fatty acids to the small intestine in steers

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**Introduction** Previous studies have shown that including fish oil (FO) in the diet of beef cattle resulted in increased long chain C20*n*-3 PUFA (C20:5*n*-3 and C22:6*n*-3) in muscle resulting in a lower *n*-6:*n*-3 ratio (Scollan *et al*., 2005). Fish oil is considered to be a good inhibitor of biohydrogenation in the rumen, resulting in increased production of C18:1 *trans*-11 (Vaccenic acid), the precursor for conjugated linoleic acid (CLA *cis*-9, *trans*-11) in muscle. This study investigated the effects of incremental levels of FO in the diet on fatty acid metabolism in the rumen.

**Materials and methods** Six Hereford × Friesian steers (mean live weight 609 kg ± 6.9), prepared with rumen and duodenal cannulae were used in a replicated Latin square experiment. The treatments were based on grass silage plus one of three concentrates in which the level of FO (salmon oil) was 0, 20 or 60 g/kg fresh, referred to as Control, FO1 and FO2, respectively. Total feed intake was offered at 14 g dry matter (DM)/kg live weight with the ratio of forage : concentrate at 60:40 (DM basis). All diets were designed to be iso-nitrogenous and iso-energetic with total lipid made up to 6% DM intake. Total-N and acid hydrolysis ether extract averaged 26.0 and 65.2 g/kg DM across all three concentrates. On days 16 and 17 of each 21 d period duodenal digesta was collected manually every 3 h over a 24 h period, bulked and freeze-dried for chemical analysis, and a dual-phase marker technique with Cr-EDTA and Yb-acetate was used to assess digesta flow. The analysis of fatty acid methyl esters were as described by Lee *et al*. (2003). Statistical analysis was undertaken by ANOVA (GenStat 8th edition).

**Results** Dry matter intakes were similar across treatments and averaged 8.28 kg/day (s.e.m. 0.027). Increasing the level of FO resulted in greater intake and flow of EPA, DPA and DHA (P<0.01). Stearic acid (C18:0) flow to the duodenum was much higher than intake of C18:0 and was much reduced with increasing from Control to FO2 (P<0.001). Flow of C18:1 *trans*-11 increased 1.6 fold on FO2 relative to the Control (P<0.001). Flow of C18:1 trans-10 also increased (P<0.001). Biohydrogenation of C18:2*n*-6 and C18:3*n*-3 averaged 0.91 and 0.96, respectively, across treatments.

**Table 1** Intake and flow of fatty acids to the small intestine of steers given the experimental diets (g/day)

| | Control | FO1 | FO2 | s.e.m. | P |
|---|---|---|---|---|---|
| Fatty acid intake | | | | | |
| Total fatty acids | 409 | 396 | 388 | | *** |
| 18:0 stearic | 11.2 | 10.3 | 8.7 | 0.10 | *** |
| C18:2 *n*-6 linoleic | 73.7 | 68.8 | 65.6 | 0.32 | *** |
| C18:3 *n*-3 α-linolenic | 80.8 | 81.2 | 83.1 | 0.37 | *** |
| C20:5 *n*-3 eicosapentaenoic (EPA) | ND | 3.50 | 13.99 | 0.090 | *** |
| C22:5 *n*-3 docosapentaenoic (DPA) | ND | 0.89 | 3.59 | 0.024 | *** |
| C22:6 *n*-3 docosahexaenoic (DHA) | ND | 4.76 | 20.10 | 0.128 | *** |
| Fatty acid flow to small intestine | | | | | |
| Total fatty acids | 402 | 386 | 316 | 11.1 | *** |
| C18:0 stearic | 162.9 | 122.9 | 55.8 | 6.83 | *** |
| C18:1 *trans*-11 | 16.9 | 29.0 | 44.3 | 2.95 | *** |
| C18:1 *trans*-10 | 2.8 | 3.6 | 15.3 | 1.1 | *** |
| C18:2 *n*-6 linoleic | 7.4 | 6.9 | 3.8 | 0.48 | *** |
| C18:3 *n*-3 α-linolenic | 3.4 | 3.3 | 2.2 | 0.25 | * |
| CLA *cis*-9, *trans*-11 | 0.18 | 0.24 | 0.16 | 0.022 | 0.083 |
| C20:5 *n*-3 eicosapentaenoic (EPA) | 0.27 | 0.44 | 0.81 | 0.063 | *** |
| C22:5 *n*-3 docosapentaenoic (DPA) | 0.23 | 0.33 | 0.87 | 0.101 | ** |
| C22:6 *n*-3 docosahexaenoic (DHA) | 0.19 | 0.38 | 0.95 | 0.087 | *** |

*, ** and *** indicate P<0.05, P<0.01 and P<0.001, respectively. ND = not detected.

**Conclusions** Feeding increasing amount of FO in the diet resulted in increases in the flows of EPA, DPA and DHA to the small intestine which explains the increased deposition of these PUFA in muscle lipids (Scollan *et al*., 2005). The large reduction in C18:0 flow to the duodenum and increased of C18:1 *trans* supports to the concept that FO inhibits the final step in the biohydrogenation pathway, from C18:1 *trans*-11 to C18:0. FO also increased the *trans*-10 pathway as evident in the increased flow of C18:1 *trans*-10.

**Acknowledgements** This work was supported by Department for Environment Food and Rural Affairs.

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# Evaluation of progeny from beef bulls with either a Top 1% or Top 10% Beef Value

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**Introduction** In a previous study at Harper Adams University College (Marsh & Pullar 2002) the progeny from bulls with either a high (Top 10% Beef Value [LM29]) or below average Beef Value (Bottom 25% Beef Value [LM7]) were reared through to slaughter on a silage beef system. The calves sired by the Top 10% Beef Value bull recorded significantly higher carcass weights. The objective of this trial was to evaluate the performance of Limousin cross Holstein Friesian calves sired by bulls with either a Top 1% Beef Value or Top 10% Beef Value.

Materials and methods In April 2004 Holstein-Friesian dairy cows were inseminated with semen from the Limousin bulls; Killerton Travis (Top 1% Beef Value of LM39) and Cockleshell Olympus (Top 10% Beef Value of LM30). The calves were born from January to March 2005 and reared through to slaughter on a cereal beef system with 13 bulls and 13 heifers per sire. The cattle were housed in straw-bedded pens and fed a 150g CP/kg proprietary beef nut (Carrs Billington) *ad libitum*. The bulls were selected for slaughter at MLC fat class 3 and heifers at fat class 4L (EUROP carcass classification: Conformation: P+=1 and E=7, Fat class: 1=1 and 5H=7). The data was analysed using ANOVA.

**Results** The calves sired by Killerton Travis (Top 1% Beef Value sire) recorded significantly higher ($P<0.05$) daily liveweight gains (DLWG), daily carcase gain, slaughter weights, carcase weights, improved carcase conformation scores and carcase values compared to calves sired by Cockleshell Olympus (Top 10% Beef Value). The Killerton Travis bred cattle also recorded a higher killing out percentage but this was not statistically significant. 'Beef Value' is an assessment of the economic genetic merit of an animal. The theoretical difference between the progeny from the sires should have been £4.50. In this study the difference in financial value was £29.70 per calf thus exceeding the predicted value by £25.20. This simplistic calculation does not take into account the possible benefits accruing through the reduced incidence of calving difficulties and reduced number of days to reach slaughter condition with Killerton Travis which should further increase the financial benefit for this Top 1% Beef Value sire.

**Table 1** Animal Performance (bulls & heifers)

| | Top 10% | Top 1% | s.e.d | Sig |
|---|---|---|---|---|
| Gestation (days) | 289.3 | 287.8 | 1.40 | NS |
| Calving ease[1] | 1.58 | 1.21 | 0.148 | NS |
| Birth weight (kg) | 48.5 | 48.3 | 0.90 | NS |
| Slaughter weight (kg) | 533.5 | 551.7 | 8.63 | * |
| Days to slaughter | 396.5 | 393.8 | 6.63 | NS |
| DLWG (kg) | 1.22 | 1.28 | 0.028 | * |
| Carcase wt (kg) | 294.3 | 307.0 | 5.35 | * |
| Kill out (g/kg) | 551 | 555 | 0.371 | NS |
| Carcase daily gain (kg) | 0.68 | 0.72 | 0.016 | * |
| Conformation class | 3.67 | 4.00 | 0.155 | * |
| Fat class | 3.67 | 3.63 | 0.136 | NS |
| Carcase price (£/kg) | 1.93 | 1.95 | 0.009 | NS |
| Carcase value (£) | 568.3 | 598.0 | 11.78 | * |

[1] Calving Ease: 1 = unassisted, 5 = caesarian,

**Table 2** Animal Performance (bulls v heifers)

| | Top 10% | | Top 1% | | |
|---|---|---|---|---|---|
| | Bulls | Heifers | Bulls | Heifers | s.e.d |
| Birth weight (kg) | 50.4 | 46.6 | 49.8 | 46.7 | 1.28 |
| Slaughter weight (kg) | 579.1 | 487.9 | 597.9 | 505.5 | 12.21 |
| Days to slaughter | 403.6 | 389.3 | 401.4 | 386.2 | 9.37 |
| DLWG (kg) | 1.30 | 1.15 | 1.37 | 1.19 | 0.040 |
| Carcase wt (kg) | 327.3 | 261.3 | 341.2 | 272.8 | 7.57 |
| Kill out (g/kg) | 565 | 535 | 571 | 540 | 0.524 |
| Carcase daily gain (kg) | 0.75 | 0.62 | 0.80 | 0.65 | 0.023 |

There were no significant sire by sex interactions.

**Conclusions** The performance of the bulls and heifers exceeded the recognised targets for cereal fed Continental cross beef cattle. The majority of the results from this experiment tend to confirm that Estimated Breeding Values can provide a reasonably accurate measure of an animal's genetic merit, however in this experiment they significantly under predicted the financial benefit that can accrue from using a bull with a Top 1% Beef Value compared to a bull with a Top 10% Beef Value.

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# Genetic evaluation of animals with and without using genotypic data of major gene loci

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**Introduction** Now a days, scientists like to find about association of major genes and quantitative traits. In the first step, breeding value of quantitative trait should be predicted and genotype of animals for special major gene locus should be detected. Then, GLM analyses are used to compare all levels of genotypes and study about their association with quantitative traits. The accuracy of prediction of breeding value may influence the result of analyses. Different models with different accuracy of prediction may be utilized to predict breeding value. In this article, different models, with and without using the Genotypic Data of Major Genes Loci were used in order to identify the better model for genetic evaluation in this situation.

**Materials and methods** A single major gene with two alleles (A and a), frequency p and q, genotypic value +a (AA), d (Aa) and –a (aa) with breeding value 2qα (AA), (q-p)α (Aa) and -2pα (aa) was simulated. Also $\alpha = a + (1-2p)d$ and $\sigma^2_{AQ}=2pq\alpha^2$ where $\sigma^2_{AQ}$ is additive genetic variance explained by the major gene and α is average allele substitution effect(Dekkers *et all.,* 1999. Falconer and Mackay., 1996). It was assumed that $\sigma^2_{AQ}$ is a fraction of total additive genetic variance ($\sigma^2_u$ ) and equal to 0.15, 0.25, and 0.35 in different runs of the program and the initial frequency of the favourable allele (A) equal to 0.2. Then all the above values were simulated. We assumed that all animals of the base population were genotyped for major gene locus and the major gene alleles transmitted from parents to offspring in classical Mendelian fashion. The polygenic effect for each animal was obtained from a normal distribution with mean 0 and variance $\sigma_{APoly}$. Also $P_i = TBV_i + e_i$ , $TBV_i = BV_{polyi} + BV_{Qi}$ where $P_i$ is the phenotypic value, $TBV_i$ is the total genetic value, $BV_{polyi}$ is the breeding value of the polygene and $BV_{Qi}$ is the breeding value of major gene in the animal i. $e_i$ is the environmental deviation and obtained from a normal distribution with mean 0 and variance $\sigma_e$ (Chakraborty *et all.,* 2002, Villanueva *et all.,* 2004).The polygenic effect of offspring was generated as the avarege of the polygenic effect of the parent plus a Mendelian sampling. Then the data file was prepared and genetic evaluation was done by DFREML with two different models ($y_i= u+g_i+A_i+e_i$) (model 1) and ($yi= u +A_i+e_i$) (model 2) where $y_i$, $A_i$ and $g_i$ are the phenotype, additive genetic value and genotype of the animal i respectively and u is the overall mean. The correlation between estimated breeding values (breeding value estimated by DFREML with 2 models) with true breeding value (simulated breeding value) was calculated. Also the real heritability and estimated heritability (estimate by model 1 and 2) were compared. These criterions were used to define which model is better.

**Results** Table 1 shows that with increasing the effect of major gene than polygene (which affects the quantitative traits) the amount of $h^2$ decreases but the correlation between TBV and EBV increases. The amount of heritability and correlation between TBV and EBV shows that model 2 gives a more accurate result. So using model 2 is better than model 1.

Table 1:comparision of models 1 and 2 with consideration of heritability and Correlation between TBV and EBV

| Fraction of $\sigma^2_{AQ}$ in $\sigma^2_u$ | Heritability* | | | Correlation between TBV &EBV | |
|---|---|---|---|---|---|
| | Real | Model 1 | Model2 | Model 1 | Model2 |
| 15 | 0.34 | 0.27 | 0.33 | 0.59 | 0.78 |
| 25 | 0.31 | 0.21 | 0.30 | 0.70 | 0.81 |
| 35 | 0.28 | 0.14 | 0.26 | 0.77 | 0.83 |

*Standard error of Heritability for model 1 and model 2 equals to 0.007

**Conclusions** The inheritance of major gene is like a qualitative traits so with increasing the effect of major gene the ratio of polygenic variance and total genetic variance, that transmit to the next generation, decrease. So heritability will be decreased. Also REML predicts total breeding value but all data were corrected for genotype of major gene in each individual in model 1. Furthermore, major gene breeding value is apart of total breeding value of quantitative trait hence model 2 gives better results than model 1.

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# Using Wood's function to estimate phenotypic correlations among lactation curve parameters in Iranian first lactation buffaloes

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**Introduction** In dairy farm animals, production of milk and its components varies in a curvilinear pattern over the course of the lactation. Knowledge of the lactation curve may provide a worthwhile information source about the pattern of milk production which in turn could be used for herd management decisions. Moreover, inter-relationships among lactation curve parameters could be utilised in applied animal breeding programmes to change more effectively the shape of the lactation. Many studies have been undertaken to apply different mathematical models to obtain more accurate prediction of the shape of the lactation curve among which the incomplete gamma function first proposed by Wood (1967) has been broadly used by previous research workers (Rekaya *et al*., 2000; Tekerli *et al*., 2000). The main objective of the present research was to apply Wood's function for estimating phenotypic correlations among lactation curve parameters in Iranian first lactation buffaloes.

**Material and methods** A total of 7426 monthly test day milk yields collected from 1142 Iranian buffalo heifers calving between 1993 and 2005 and distributed in 383 herds was used to estimate phenotypic correlations among lactation curve parameters. Wood's incomplete gamma function ($y_t=at^b(Exp(-ct))$) was fitted to individual lactations for estimating lactation curve parameters (a, b and c) as well as some production characteristics (peak time, milk yield at peak time, persistency and total lactation milk yield) by the use of statistical analysis system (SAS). In the Wood's function, $y_t$ is milk yield at month t, a is a parameter associated with milk yield at the beginning of the lactation, t is month of the lactation, b and c are parameters associated with inclining and declining slopes of the lactation curve and Exp is equal to 2.71828. Pearson product moment correlation coefficients among the lactation curve parameters as well as production characteristics were subsequently obtained by SPSS programme.

**Results** Pearson product moment correlation coefficients among lactation curve parameters as well as production characteristics of Iranian first lactation buffaloes are given in Table 1. Initial milk yield (parameter a) had positive correlations with parameter c, peak yield and lactation milk yield while it was negatively correlated with parameter b, peak time and persistency. Parameter b had positive correlations with c and peak time and negative correlations with persistency, peak yield and lactation milk yield which are similar to the results obtained in previous studies (Tekerli *et al*., 2000). Parameter c was found to be negatively correlated with peak time, persistency, peak yield and lactation milk yield. Peak time showed positive correlations with persistency, peak yield and lactation milk yield. Milk yield at peak time was positively correlated with persistency and lactation milk yield. A positive correlation was also found between persistency and lactation milk yield. Among positive correlations, the largest and smallest correlations were found between peak yield and lactation milk yield and between peak yield and persistency respectively.

**Table 1** Pearson product moment correlation coefficients* among lactation curve parameters as well as production characteristics** in first lactation Iranian buffaloes

| Trait | a | b | c | PT | PY | Per | Milk |
|---|---|---|---|---|---|---|---|
| a | 1.00 | -0269 | 0.126 | -0.444 | 0.743 | -0.360 | 0.590 |
| b | | 1.00 | 0.787 | 0.291 | -0.014 | -0.352 | -0.086 |
| c | | | 1.00 | -0.191 | -0.020 | -0.798 | -0.182 |
| PT | | | | 1.00 | 0.047 | 0.678 | 0.187 |
| PY | | Symmetric | | | 1.00 | 0.025 | 0.909 |
| Per | | | | | | 1.00 | 0.237 |
| Milk | | | | | | | 1.00 |

* Most correlations were significant at $p<0.001$.
**Peak time (PT) calculated as b/c, peak yield (PY) calculated as $a(b/c)^b(Exp(-b))$ and persistency (Per) calculated as $(-(b+1)Ln(c))$.

**Conclusion** The results found in the present research indicated that milk yield at peak time of the lactation was highly positively correlated with total lactation milk yield suggesting that the former could be used in replace of the latter one in selecting animals to decrease generation interval which in turn could result in increasing genetic gain per year. Positive correlation between parameters b and c indicates that cows with lower inclining slope would expect to have lower declining slope over the course of the lactation. Positive correlations among peak time, persistency and total lactation milk yield shows that selection for later peak time could result in higher persistency and greater amount of lactation milk yield.

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# A missense mutation in the bovine leptin gene in Iranian Taleshi cattle

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**Introduction** Candidate gene approaches facilitate discovering and localizing causative genes for quantitative traits and polymorphisms within selected candidate genes can be tested for association with variationin the quantitative trait (Campbell et al., 2003). Leptin is a 16-kDa protein that is synthesized and secreted predominantly by white adipocytes tissue and its expression is regulated by body fatness and energy balance. Leptin gene expressed in a variety of tissues including adipose tissue, placenta, mammary glands, skeletal muscles, gastric mucosa, brain and pituitary glands. In cattle, the leptin gene is located on chromosome 4 and consists of three exons .Several studies shown the association between polymorphism at the leptin gene and feed intake, energy balance, fertility and immune functions. It has been shown that leptin gene influences milk performance in cattle and reproduction in beef cattle(Liefers et al., 2002). Buchanan et al. (2002) described a cytosine (C) to thymine (T) substitution in exon 2 of the leptin gene of the *B. taurus* breeds, suggesting the existence CC, TT and CT genotypes. The aim of this study was to identify the single nucleotide polymorphism of exon 2 in Iranian Taleshi cattle.

**Materials and methods** Blood samples were collected from 64 Taleshi cattle from Talesh city in Guilan province, Iran. DNA was extracted from 100 micro litters whole blood by Guanidinium Thiucianate-silica gel method. Quality and quantity of extracted DNA were measured by Biometra UV photometer. One µl DNA (50 ng) was amplified in a total volume of 20 µl PCR mix using the Biometra T Personal thermocycler Ver: 1.11. The PCR mixture contained: 2.5 µl PCR buffer 10-X (200 mM $(NH_4)_2SO_4$, 0.1 mM 750 mM Tris-HCl pH=8.8), 2.5 mM $MgCl_2$, 2 mM dNTPs, 2 µl mixture of primers (10 pM from each primer), 1 u *Taq* DNA polymerase and 12 µl ddH2O. Samples were amplified for 34 cycles at the following program: initial denaturation step of 4 min at 94 º C followed by 35 cycles 45 s at 94 º C, 45 s at 58 º C, 45 s at 72 º C and final extension step of 8 min at 72 º C. The sequences of primers (AF120500 accession number) were: 5'ATGCGCTGTGGACCCCTGTATC 3' and 5'- TGGTGTCATCCTGGACCTTCC–3', that amplified a 94 bp fragment from the exon 2 of bovine leptin gene. Products of amplification were recognized by electrophoresis on 2% agarose gel stained with etithium bromide. The PCR products were digested with 3 units of *Bsp*13I enzyme for 6 hours in 50º C. Digestion fragments were revealed by 3% agarose gel stained with etithium bromide. The frequencies of alleles and genotypes were calculated by Popgene 1.32 software.

**Results** Restriction Fragment length polymorphism (RFLP) analysis revealed two different alleles: allele T was a 94bp fragment and C was two fragments of 75 and 19 bp. The allele frequencies of T and C were 0.45 and 0.55, respectively. Three genotypes of TT, TC and CC were found with the respective frequencies of 0.27, 0.36 and 0.37. The observed, expected and average heterozygosity were 0.359, 0.498 and 0.494, respectively (Table1). The $\chi^2$ test ($P<0.05$) confirmed that Hardy-Weinberg equilibrium existed in this population.

Table1.The allelic and genotypic frequencies, observed, expected, Nei's expected and average heterozygosity and $\chi 2$ test for the exon 2 of the leptin gene.

| allelic frequencies | | genotypic frequencies | | | Obs_Het | Exp_Het* | Nei** | Ave_Het | $\chi 2$ |
|---|---|---|---|---|---|---|---|---|---|
| T | C | TT | TC | CC | | | | | |
| 0.45 | 0.55 | 0.27 | 0.36 | 0.37 | 0.359 | 0.498 | 0.494 | 0.494 | 5.03 |

* Expected homozygosity and heterozygosity were computed using Levene (1949)
** Nei's (1973) expected heterozygosity

**Conclusions** The results of this study showed that there was large variability in this breed. This variability Could be regarded as a useful tool for selection programming, mainly marker associated selection between different genotypes of the presented loci.

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# Association of genetic variants of the prolactin gene with milk production traits in Russian Red Pied cattle

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**Introduction** Prolactin plays an important regulatory function in mammary gland development, milk secretion, and expression of milk protein genes. Hence the PRL gene is a potential genetic marker of production traits in dairy cattle. The gene was mapped on chromosome 23 by Hallerman et al. (1988). It consists of 5 exons and four introns (Camper et al. 1984) encoding the 199-amino-acid mature protein (Wallis 1974). On the basis of sequence analysis of four different cDNA clones, seven possible nucleotide substitutions were described by Sasavage et al. (1982). One of them, recognized by RsaI endonuclease, has become a popular genetic marker used for genetic characterization of cattle populations by means of PCR-RFLP (Mitra et al., 1995). Two allelic variants (B and b) have been distinguished at the DNA level, based on RsaI polymorphism in the third exon of the coding region. It has been suggested that prolactin alleles correlate with milk yield (Lewin et al., 1992).

**Materials and methods** A total of 98 Red Pied cows were genotyped. The cows were kept in the Drydjba herd in Varonedj state of Russia. The PRL-Rsal genotypes were analysed using the PCR-RFLP method. PCR Primer Sequences was amplified using forward (5'-CGAGTCCTTATGAGCTTGATTCTT-3') and reverse (5'-GCCTTCCAGAAGTC GTTTGTTTTC-3') primers. Cycles applied were: denaturation - 94°C/5 min, followed by 30 cycles - 94°C/30 s, primer annealing - 59°C/40 s, PCR products synthesis - 72°C/20 s, and final synthesis - 72°C/3 min. PCR Conditions: 2,5 µl 10 x PCR buffer( 15 mM $MgCl_2$) 1,5 µl dNTP-mix (2 Mm each), 1.5 µl of primer(100 pmol/µl each), 0/5 u Taq Amplified DNA was digested with Rsal enzyme. Digestion products were separated electrophoretically in 4% agarose gel. Data for 305-day milk production, including overall yields of milk, milk fat and milk protein, percent of milk fat and percent of milk protein and combined milk fat and milk protein percent were obtained from the farm records. Statistical calculations were performed using general linear models (GLM) procedure of SAS. Frequencies of distribution of alleles within the herds were compared using the Chi-square test. The effect of PRL genotypes on the milk production traits of cows was analysed using GLM procedure.

**Table 1** Effect of prolactin genotypes on the milk traits in Russian Red-pied cows

| Genotype | Fat% ±SD | Protein% ±SD | Fat(kg)± SD* | Protein(kg) ± SD | Milk(kg)±SD** |
|---|---|---|---|---|---|
| AA | 3.58±0.63 | 3.27±0.09 | 247.29[a]±46.30 | 248.55[a] ±26.56 | 6709.24[a]±1328 |
| AB | 3.71±0.52 | 3.18±0.60 | 236.54[b]±55.92 | 241.17[a]±28.88 | 6182.38[b]±1511 |
| BB | 3.63±0.39 | 3.23±0.01 | 260.03c±35.65 | 279.69[b]±10.00 | 7239.00[c]±1504 |

*Significant at P<0.05, ** Significant at P<0.01.
[ab] Within column mean values are different (P<0.05)

**Results** In this breed the frequencies of alleles were as follows; A= 0.794 B= 0.206, respectively. The frequencies of AA, AB and BB genotypes were 0.598, 0.392 and 0.01; respectively. And $\chi^2$=0.034 ≤ 3.84. Table 1 shows the effect of the PRL-Rsal polymorphism on milk production traits in cows studied. Milk yield**.** BB genotype BB had higher milk yield (+529.76 kg and +1056.61 kg, respectively) than AA and AB individuals. Differences (P<0.05) between the cows with different PRL-Rsal genotypes were observed in milk fat yield with BB cows showing higher (P < 0.05)milk fat yield (+12.74 kg and +23.49 kg, respectively) than AA and AB individuals. AA cows yielded more milk fat (+10.75 kg) than AB animals (P < 0.05). There was no difference in milk fat concentration between the cows with different PRL-Rsal genotypes (P > 0.05). Similarly, no differences in milk protein concentration existed between cows of different PRL-Rsal genotypes (P > 0.05). However, cows with the BB genotypes, produced more milk protein (31.14 kg and 38.52 kg, respectively) than AA and AB individuals (P < 0.05) and cows with AA genotype produced more milk protein (+7.38 kg) than cows with AB genotype (P < 0.05).

**Conclusions** The results show that the highest milk and milk fat yield were obtained by cows with genotype PRL-Rsal BB. The results presented here show that the prolactin gene may be considered as a marker for dairy traits in cattle

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# Estimation of genetic and phenotypic parameters for lactation traits of buffaloes in Khuzestan province in Iran

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**Introduction** Buffaloes have important role in Khuzestan agricultural economy in Iran. They are adapted to harsh environmental conditions in the area. They are well resistant against ticks and disease and used to eat low quality feeds. In order to establish a breeding plan, estimation of genetic parameters is necessary. The objective of this study was to explore production and genetic capacity of Khuzestan' buffalos.

**Materials and methods** A total of 7583 milk and lactation records, 7547 fat yield and fat percentage records in 228 buffaloes herds during 1993 to 2003 were collected by Animal husbandry centre of Jahad-Agriculture Organisation in Khuzestan province. Data were manipulated for deletion of outlying records. Primary analysis of data were performed using SAS program in order to predict effects of herd, parity, year and season of calving on milk and fat yield, fat percentage and the length of lactation periods. The structure of data is presented in Table 1. Genetic parameters (heritability, genetic and phenotypic correlation) were estimated using Restricted Maximum Likelihood method by Animal Model of DFREML software (Meyer, 1998).

**Table 1** Structure of raw data.

| Traits | No. of records | Average | Min. | Max. | SD | CV |
|---|---|---|---|---|---|---|
| Milk yield (kg) | 7583 | 2085.13 | 345 | 7065 | 826.25 | 39.63 |
| Fat yield (kg) | 7547 | 129.47 | 13 | 446 | 59.66 | 46.08 |
| Fat (%) | 7547 | 5.67 | 2 | 9 | 1.13 | 19.99 |
| Days in milk | 7583 | 218.2 | 74 | 786 | 71.57 | 32.80 |

**Results** Primary analysis showed that the effects of herd, year and season were highly significant on all of the considered traits ($P<0.01$). While parity effects was only significant on milk and fat yield ($P<0.05$). There are very good variation among buffaloes which is a good tool for selection (Table 1). Amount of variation for fat yield is more than the other considered traits according to calculated CV (46.08) followed by milk yield (CV=39.63). Average of days in milk was 218 .2 days in 228 herds ranging from 74 to 786 days. It may be largely due to different management methods and environmental condition employed. Heritability of milk yield, fat yield and fat percentage were high for all of the parities using multi-trait animal model (Table 2).

**Table 2** Heritability (diagonal) genetic (above diagonal) and phenotypic (below diagonal) correlation of milk yield traits of Khuzestan's buffaloes

| Trait | Milk yield parity (MYP) | | | Fat yield parity (FYP) | | | Fat percent parity (FPP) | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1st | 2nd | 3rd | 1st | 2nd | 3rd | 1st | 2nd | 3rd |
| 1st MYP | <u>0.48</u> | 0.45 | 0.68 | 0.83 | 0.73 | 0.77 | -0.13 | -0.12 | -0.12 |
| 2nd MYP | 0.46 | <u>0.47</u> | 0.45 | 0.64 | 0.84 | 0.78 | -0.08 | -0.07 | -0.07 |
| 3rd MYP | 0.59 | 0.46 | <u>0.38</u> | 0.63 | 0.50 | 0.72 | -0.13 | -0.12 | -0.12 |
| 1st FYP | 0.85 | 0.50 | 0.62 | <u>0.56</u> | 0.91 | 0.96 | 0.10 | 0.95 | 0.11 |
| 2nd FYP | 0.67 | 0.64 | 0.51 | 0.82 | <u>0.59</u> | 0.93 | 0.55 | 0.05 | 0.06 |
| 3rd FYP | 0.70 | 0.56 | 0.73 | 0.84 | 0.78 | <u>0.57</u> | 0.09 | 0.08 | 0.09 |
| 1st FPP | -0.13 | -0.10 | -0.12 | 0.05 | 0.05 | 0.06 | <u>0.65</u> | 0.80 | 0.80 |
| 2nd FPP | -0.12 | -0.10 | -0.11 | 0.06 | 0.05 | 0.06 | 0.72 | <u>0.77</u> | 0.73 |
| 3rd FPP | -0.13 | -0.01 | -0.12 | 0.06 | 0.05 | 0.07 | 0.72 | 0.75 | <u>0.77</u> |

**Conclusions** There was a high variation among recorded buffaloes in Khuzestan province as well as high heritability for considered traits which expect selection procedures effective. Low genetic and phenotypic correlation between milk yield and fat percentage shows that these traits could improve independently. Overall, results shows that by good selection programme and improving management including nutrition, health care and reproduction traits one could improve milk and other production traits.

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# Study of genetic and environment trends for milk fat yield trait in an Iranian dairy herd

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**Introduction** In order to evaluate genetic improvement in a selected population, variation resulted from environment and genetics should be dissociated. Because of the positive genetic correlation between milk fat yield and milk and protein yield, selection for milk production has often resulted in an increase in fat and protein yields however response for fat percentage is negative. However positive genetic trends between 0.73 to 0.95 kg per annum for milk fat yield in Holstein cattle have been reported in some studies that milk fat yield had less importance in selection index, also negative values are reported. Based on previous research, genetic improvement of milk fat yield up to 1 percent due to selection is possible and from a theoretical view, an improvement of about 2 percent is reported. With attention to selection in the herd and using progeny tested semen from foreign countries that have suitable genetic potential, this study was accomplished in order to estimate genetic parameters for milk fat and investigate genetic and environmental trends during 1990 to 2001.

**Materials and methods** Milk fat yield data from pedigree Holstein herd was used. This data set included first and second lactation records, collected during 1990-2001 by Khorasan Animal Breeding Centre. This data set was obtained from 114 and 801 sires and their daughters respectively. The records were corrected based on 305 day of lactation and twice daily milking. For prediction of genetic parameters, two models were used: the unvaried model was applied to first lactation records and the repeatability model was applied to first and second lactation records. (Co) Variance components were estimated for milk fat yield, using an animal model which was applied to first and second lactation records. The method was based on a derivative-free algorithm. Variance components and genetic parameters were estimated by DFREML software. Models obtained were:

$y_{ijk}= \mu+ (YS)_i + a_j + b_1D_{jk} + b_2D^2_{jk} + b_3D^3_{jk} + e_{ijk}$

$y_{ijk}= \mu+ (YS)_i + a_j + P_i + b_1D_{jk} + b_2D^2_{jk} + b_3D^3_{jk} + e_{ijk}$

*$y_{ijk}$=Observation; $\mu$=Trait mean; $(YS)_i$=Effect of year season(fixed );*

*$a_j$=Genetic effect of j animal(random); $b_1,b_2,b_3$=Linear ,Quadratic, cubic Regression Coefficient of gestation age; $D_{jk}$=K age group of j animal (covariate); $P_i$= Permanent environment effect $e_{ijk}$=Residual effect (Random)*

Genetic trend of milk fat yield was estimated by regressing average breeding values (BV) on gestation year (in sires, on gestation year of their daughters).

**Results** Estimated genetic and environmental trends for milk fat yield is shown in figure 1. Obtained results of genetic trend estimation by two noted models were the same. Estimated genetic trend for milk fat yield of sires and cows were -0.420±0.292 (b±cv) and -0.161±0.067kg respectively. Mean of milk fat yield in cows increased by 9.38+0.72kg per year and estimated environmental trend of milk fat yield of the cows was 9.52 kg per year displaying an improvement and environmental condition in different years.

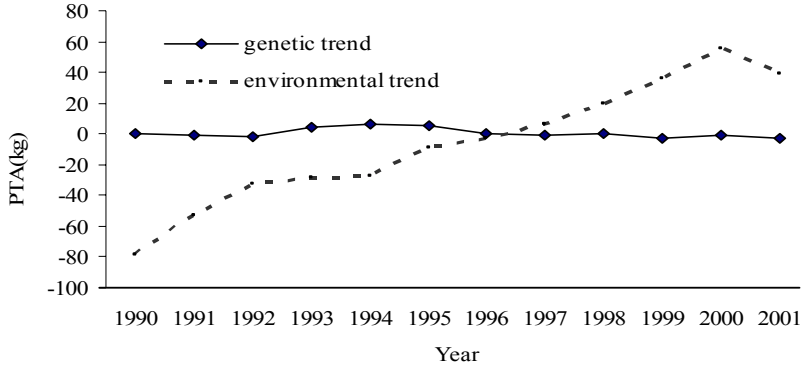


**Figure1** Estimated genetic and environmental trends for milk fat yield(kg)

**Conclusions** The results indicate that selection and mating in the herd were only based on phenotype, not BV. It must be noted that the semen from other countries (The USA and Canada) were used, so factors such as interaction between genotype and environment can reduce genetic improvement. In other words, because of interaction between genotype and environment, animals that are superior in an environment possibly may not be superior in other environments and may have poorer performance.

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## Sequence determination of 16s ribosomal RNA for ruminal ammoniabacters of Holstein cows

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**Introduction** Protein degradation in the rumen often proceeds at a rate which exceeds the ability of the microbial population to utilize the resulting breakdown products. Bacteria are the most active proteolytic organisms in the rumen, and many species of rumen bacteria are known to be proteolytic (Wallace *et al*., 1985). The commonly isolated proteolytic bacteria are also able to break down peptides and amino acids, and it was assumed that they were responsible for the ruminal degradation of intact protein through to ammonia. However, studies comparing the specific activities of ammonia production between mixed ruminal bacteria and the well-known proteolytic bacteria noted that no individual bacterium had an activity which could explain the activity of the mixed ruminal culture (Russell *et al.,* 1988). Subsequently, three gram-positive, monensin-sensitive, ammonia hyper producing (HAP) bacteria were isolated from the rumen. The present study reports the sequence of 16sr RNA for ruminal ammoniabacters of Iranian Holstein cows.

**Materials and methods** Rumen samples were collected from 10 fistulated mid lactation Holstein cows (30±3.6 milk production) fed 19 kg dry matter of a diet (58:42 forage:concentrate) containing 18% CP and 1.56 Mcal/kg DM NEL. Rumen fluid was centrifuged for 10 min in 3000rpm then DNA was extracted from 200 µl of supernatant by Guanidinium Thiocyanate-Silica Gel method (Boom et al., 1990).
In a volume of 20 µl which consisted of 50 ng of template DNA, PCR reaction contained: 2.5 µl PCR buffer 10-X (200 mM $(NH_4)_2SO_4$, 0.1 mM Tween 20, 750 mM Tris-HCl, and pH=8.8), 2.5 mM $MgCl_2$, 200 µM dNTPs, and 10 pM of oligonucleotids, 1 U *Taq* DNA polymerase. Thermal condition consisted with thirty-five cycles of 95°C (1 min), 62°C (1 min), and 72°C (2 min) followed by 72°C (8 min). 16sRNA gene was amplified to a product of 1500 bp fragment using the primers described by Attwood et al. (1998). Primer sequences were FD1: 5'-GAGTTTGATCCTGGCTCAG-3' and RD1: 5'-AAGGAGGTGATCCAGCC-3'.
PCR products were visualized by electrophoresis on 1% agarose gel stained with etithium bromide. PCR product after purification was sequenced for analysis of 16s ribosomal RNA. The obtained sequence was analyzed with Chromas Lite 2.01 software and aligned with BLAST 2.0 for finding similarity with other sequences.

**Results** Extracted DNA had a good quality and 1500 bp fragments of 16s rRNA from ammoniabacters and fibrobacters was amplified successfully (Figure 1). Sequence of purified PCR product showed a high homology with other sequences with accession number of DQ394612, DQ394611 and DQ394610.

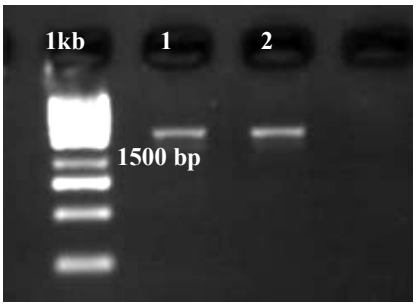


**Figure 1** Agarose (1%) electrophoresis of 1500 bp fragment of 16s rRNA (Lanes 1 and 2). DNA Ladder is 1Kbp (Fermentas).

**Conclusions** This is the first study to report the sequence of 16s ribosomal RNA for ruminal ammoniabacters. Phylogenetic study of ruminant bacteria is very important for detection and modification of rumen flora. Such a sequencing method is suitable for detecting bacteria. For exact determination in experimental an industrial purposes, we recommend specific culture for special bacteria.

**Acknowledgements** This investigation was supported by Excellence Centre for Animal Science, Agriculture Faculty, Ferdowsi University of Mashhad.

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# Association of prolactin polymorphism with milk fat content in Iranian Sarabi cows

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**Introduction** Prolactin (PRL) is a polypeptide hormones produced by cells of the anterior pituitary. The bovine PRL gene consists of 4 introns and 5 exons, located on chromosome 23. Different biological functions of PRL were subdivided into five broad categories: reproduction, osmoregulation, growth, integument, and synergism with steroids. Also, It has been shown to be important for control of mammary growth, lactogenesis and lactation (Skinkyte *et al.,* 2005). Chung et al, (1996) showed that PRL-*Rsa*I locus had a significant effect on milk fat percent in dairy cattle. Therefore the PRL gene was chosen as a candidate gene for milk traits in dairy cattle. The objective of this study was to evaluate the association of genetic differences in bovine PRL gene and milk fat content in Iranian Sarabi cows.

**Materials and methods** Genomic DNA samples were obtained from 95 Sarabi cattle. DNA was extracted from whole blood by guanidinium thiocyanate-silica gel method (Boom *et al*., 1990). In a volume of 20 µl which consisted of 50 ng of template DNA, PCR reaction contained: 2.5 µl PCR buffer 10-X (200 mM $(NH_4)_2SO_4$, 0.1 mM Tween 20, 750 mM Tris-HCl, pH=8.8), 2.5 mM $MgCl_2$, 200 µM dNTPs, and 10 pM of oligonucleotids, 1 u *Taq* DNA polymerase. Thermal condition consisted with thirty-five cycles of 95°C (1 min), 62°C (1 min), and 72°C (2 min) followed by 72°C (8 min). Exon 4 of the bovine PRL gene was amplified to produce a 156 bp fragment using the primers described by Skinkyte *et al.* (2005). PCR products (4 µl) of each PCR reaction were incubated for 5 h at 37 °C with 4 µl *Rsa* I enzyme. Digestion products were separated by electrophoresis on 3% agarose gel and visualized after staining with etithium bromide. The frequencies of genotypes, alleles and Hardy-Weinberg equilibrium test were calculated using PopGene32 (ver. 1.31) program. Data from previous lactations on milk fat content were analyzed by analyzing Standard Least Square within mixed models using JMP software (version 5.1; SAS Institute Inc, NC. USA). Animal was fitted as a random effect. Statistical significance was declared at $P < 0.05$.

**Results** A 156 bp fragment of the bovine PRL gene from the exon 3 was amplified successfully. Digestion with *Rsa* I differentiated alleles A and B. The digested AA genotype revealed a fragment of 156, AB genotype exhibited three fragments of 156, 82 and 74 and BB genotype had two fragments of 82 and 74 bp. The frequencies of A and B alleles were 0.73 and 0.27. The $\chi 2$ test confirmed the Hardy–Weinberg equilibrium in this population. The milk fat percent was similar among the genotypes ($P = 0.17$) but tended to be lower in the BB cows compare with the AA and AB cows (Figure 1).

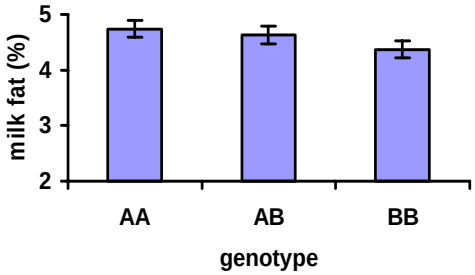


**Figure 1** Effect of the prolactin genotypes on milk fat percent using previous lactation records in Sarabi cows.

**Conclusions** The results of this study demonstrate that the prolactin polymorphism in the exon 3 had no effect on milk fat content in Sarabi cows and hence the polymorphism cannot be a suitable marker in selection programs for improving milk fat content in Sarabi cows.

**Acknowledgements** This investigation was supported by Excellence Centre for Animal Science and Dept of Animal Science, Ferdowsi University of Mashhad.

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# Performance of new hybrids of silkworm Bombyx mori (Lep.: Bombycidae)

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**Introduction** Silkworm hybrids are produced in efforts to improve the yield of silkworm rearing, whereas these genetic characteristics greatly differ among groups of hybrids (Raju and Krishnamurthy, 1995). So, the identification and evaluation of the performance of hybrids in different seasons are very important for silkworm breeding and sericulture. In this research, we evaluated the performance of 15 new silkworm hybrids, which evolved in the Iran Silkworm Research Center from pure lines in order to select the most-production hybrids for commercial application.

**Materials and methods** Performance of 12 new hybrids including Xinhang1×Koming1, Xinhang1×Koming2, Xinhang1×Y, Xinhang2×Koming1, Xinhang2×Koming2, Xinhang2×Y, Xinhang3×Koming1, Xinhang3× Koming2, Xinhang3×Y, 101433×Koming1, 101433×Koming2, 101433×Y, and also three commercial hybrids including 31×32, 103×104, 107×110 was investigated at three rearing periods. Total hatch and rearing steps of hybrids was conducted under same conditions as farmers. Other activities and traits record was carried out under standard conditions.

**Results** In table 1 performance of three main traits (number of alive larvae, total best cocoon weight and cocoon weight of 10000 larvae)) is shown for 15 studied hybrids. Other traits are explained in the text.

**Table 1** Performance of three main traits for 15 studied hybrids†

| Hybrids | Number of alive Larvae | Total Best Cocoon Weight (g) | Cocoon Weight of 10000 Larvae (g) |
|---|---|---|---|
| Xinhang1×Koming1 | 547.08 $^{de}$ | 729.79 $^{de}$ | 18893.1 $^{bc}$ |
| Xinhang1×Koming2 | 585.83 $^{bc}$ | 708.57 $^{e}$ | 17805.1 $^{e}$ |
| Xinhang1×Y | 572.5 $^{cd}$ | 751.65 $^{cde}$ | 18104.2 $^{cde}$ |
| Xinhang2×Koming1 | 591.5 $^{bc}$ | 724.3 $^{e}$ | 17620.7 $^{e}$ |
| Xinhang2×Koming2 | 650.93 $^{a}$ | 710.46 $^{e}$ | 16421.3 $^{f}$ |
| Xinhang2×Y | 607.27 $^{b}$ | 771.37 $^{bcd}$ | 17271.2 $^{e}$ |
| Xinhang3×Koming1 | 601.42 $^{bc}$ | 721.99 $^{e}$ | 17390.3 $^{e}$ |
| Xinhang3×Koming2 | 570.91 $^{cd}$ | 710.99 $^{e}$ | 17458.1 $^{e}$ |
| Xinhang3×Y | 569.5 $^{cd}$ | 714.11 $^{e}$ | 17983.5 $^{de}$ |
| 101433×Koming1 | 529.42 $^{ef}$ | 792.92 $^{abc}$ | 19791.4 $^{a}$ |
| 101433×Koming2 | 549.08 $^{de}$ | 734.69 $^{de}$ | 18653.1 $^{bcd}$ |
| 101433×Y | 524.5 $^{ef}$ | 817.79 $^{a}$ | 19403.2 $^{ab}$ |
| 31×32 | 498.67 $^{f}$ | 804.79 $^{ab}$ | 20133.6 $^{a}$ |
| 103×104 | 516.92 $^{ef}$ | 804.29 $^{ab}$ | 19719.4 $^{a}$ |
| 107×110 | 586.67 $^{bc}$ | 738.86 $^{ab}$ | 17649.7 $^{e}$ |

†Means followed by different letters in each column are significantly different (P<0.001) by DMRT.

**Conclusions** 31×31 commercial hybrid had the highest performance. Generation effect on total economical characters except cocoon shell percentage was significant (P<0.0001). Similar results were reported by Rao *et al.* (1997). Also hybrid type effect on total characters was significant except larval duration. Sex had significant effect on cocoon weight, cocoon shell weight and cocoon shell percentage (P<0.0001). Generation and hybrid interaction was not significant for best cocoon percentage, middle cocoon percentage, larval duration, and cocoon shell percentage. Alive larvae and pupae number at Xihang2×Koming2 (650.93 and 619.42 respectively) was at highest rank and at 31×32 (498.67 and 439.42 respectively) was at lowest level. At total rearing period, cocoon weigh for 10000 larvae at 31×32 (20133.6 gr) was the highest level and Xihang2×Koming2 (16421.3 gr) was the lowest level. From obtained results, it was showed that 31×32 has the best economical performance and it is recommended that these hybrids distributed between farmers.

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# The effect of a pesticide on mulberry silkworm Bombyx mori (Lep.: Bombycidae) larvae

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**Introduction** Environmental pollutants, like pesticides, have been destructive on different aspects of life. Silkworm, as a beneficial insect, is no exception to this matter. Due to this, many problems have appeared in sericulture because of pesticide applications to cultivations, especially when mulberry trees grow next to cultivated plants. Many studies that have focused on the effect of insecticides on *B. mori* deal with toxicity, retardation of development and growth, fecundity, mortality, food utilization and economic parameters (Vassarmidaki *et al.*, 2000). However, between these studies a few documents focused on the effect of fungicide residue on silkworm growth and performances (Dutta *et al.*, 2003). Sik *et al* (1976) reported that more than 1.4% of yield reduction in sericulture is due to side effect of pesticide application. 49.4% was due to the application of different pesticides in rice filed, 21.2% in fruit garden and 12.3% in olericulture. Therefore, the present investigation deals effects of long term application of systemic insecticide, Oxydemeton-methyl(metasystox R) on some biological performance of silkworm. Because metasystox has just recently used to against of mulberry thrips in sericulture and it is necessary to study different side effects of this insecticide on silkworm.

**Materials and methods** The eggs of bivoltine hybrid silkworm (103×104), obtained from Iran Sericultural Research Centre (Rasht, Iran), were reared in the laboratory with standard rearing technique (Lim *et al*. 1990) under 25ºC with humidity 75±5% and photoperiod 16Light:8Darkness. Mulberry leaves of Ken-mochi variety up to the last instar fed the larvae. In this research 0ppm (T1), 1ppm (T2), 500ppm (T3), and 1000ppm (T4) concentrations of systemic insecticide, metasystox was used as treatments. Fourth instar larvae were divided into 4 experimental groups, including control and insecticide treatments. Each group consisted of 100 larvae with three replications. Fresh mulberry leaves were soaked in each concentration for 1 minute and then were dried in air for 20 minutes. The contaminated leaves were fed to silkworm during 1$^{st}$ day of 4$^{th}$ instar. After the treatments, some larval & cocoon parameters were recorded. 4$^{th}$ Larval duration, 5$^{th}$ larval duration, Larval mortality, Total best cocoon weight and etc. Collected data were subjected to statistical analysis of variance test to find out the low significant difference between the parameters of the normal control and treated groups. For every analysis of variance, SAS software was used.

**Results** The results were in according to following diagrams:

**Table 1** The mean comparison of some parameters between different treatments[†]

| Treatments | Characters | | | |
|---|---|---|---|---|
| | Best produced cocoons weight(gr) | Larval mortality (%) | 4$^{th}$ instar Larval nutrition duration (hr) | 5$^{th}$ instar Larval nutrition duration (hr) |
| T1 | 97.21±5.313 $^{a}$ | 17.667±3.171 $^{a}$ | 116±0.00 $^{a}$ | 175±0.00 $^{a}$ |
| T2 | 98.653±5.313 $^{a}$ | 12.667±3.171 $^{a}$ | 116±0.00 $^{a}$ | 175±0.00 $^{a}$ |
| T3 | 92.363±5.313 $^{a}$ | 20±3.171 $^{a}$ | 116±0.00 $^{a}$ | 175±0.00 $^{a}$ |
| T4 | 89.38±5.313 $^{a}$ | 13±3.171 $^{a}$ | 116±0.00 $^{a}$ | 175±0.00 $^{a}$ |

[†] Mean with the same letter are not significantly different (P<0.05)

According to table1 larval mortality, weight of best cocoons produced, nutrition period duration in 4$^{th}$ & 5$^{th}$ instar in all treatments have not different significantly (P<0.05).

**Conclusions** Metasystox could alter economical and biological characters in studied hybrid numerically. But it could not alter larval duration. These results obtained by other researchers (Dutta *et al*. 2003). Thus concluded metasystox affect on important traits in sericulture and can decrease its income among farmers. Metasystox mechanism must be investigated carefully and determined functional details.

**Acknowledgements** The present investigation was conducted with support of Iran Sericultural Research Center (ISRC).

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# Chemical composition, *in situ* degradable coefficients, and ruminal and post-ruminal dry matter and crude protein disappearances of rapeseed meal

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**Introduction** Several experiments have been conducted on the value of rapeseed meal (RSM) for dairy cows (Laarveld *et al*., 1976). The crude protein (CP) content of RSM is almost high (360-420 g $kg^{-1}$ of DM) with a good amino acid balance. The *in situ* degradability of RSM was extensively studied (Kendall *et al*., 1991). The aim of the present study was to determine the chemical composition, *in situ* degradable coefficients, and ruminal and post-ruminal dry matter (DM) and CP disappearances of an Iranian variety of rapeseed meal (SLM sp.).

**Materials and methods** Ten samples of RSM (SLM sp.) were collected from different regions of Iran (From factories located in Isfahan, Behshahr and Nishabour). Then, samples were composited and sub-samples were taken from each composite. Samples were dried using a forced-air oven at 96 °C for 48 h. All samples were ground to pass through a 2-mm screen, then, analyzed for total N (Kjeldahl method), neutral detergent fibre (NDF), and acid detergent fibre (ADF), ether extract (EE), and ash concentrations. Sodium sulphite and alpha amylase were not used in the NDF assay, and NDF was expressed as the ash free residue after extraction with boiling neutral solutions of sodium lauryl sulphate and EDTA. Dry matter and CP degradability of the RSM were measured using *in situ* technique in two fistulated Holstein steers (400±12 Kg, body weight). Animals fed a 60:40 concentrate: forage diet. The samples were weighed (6 g DM) into bags (12×19 cm) made of polyester cloth with 52 µm pore size. The bags were incubated in the rumen for 0.0, 2, 4, 8, 16, 24, 48 and 72 h. The rumen removal bags were washed then dried (58 °C for 48 h). The ruminal and post-ruminal disappearances of DM and CP of samples were determined using the mobile nylon bag procedure (Danesh Mesgaran and stern, 2005). Two Holstein steers (400 kg, body weight) fitted with rumen fistulae and T-shaped cannulae were used. The samples were weighed (1g DM) into bags (3 × 6 cm) made of polyester cloth with 50 µm pore size (12 bags per each sample). The bags were incubated in the rumen for 12 hours. After removal from the rumen, six bags were washed using cold water and dried in a forced-air oven (58 °C, 48 h) to determine rumen disappearance and other bags were inserted into the small intestine via the cannulae at the rate of one bag every 30 min. Removed bags by the voided faeces were rinsed in cold ruining water. Finally, the bags were dried in a forced air oven (58 °C, 48 h), then weighted to determine the DM disappearance. The kjeldahl technique was used for nitrogen analysis. The degradable parameters of DM and CP were determined using the equation of $P= a + b (1 – e^{-ct})$, where a=rapidly degradable fraction, b=slowly degradable fraction and c= fractional degradation rate.

**Results** Chemical composition of RSM analyzed in the present study (g/kg DM; mean with SE of mean for 10 samples) was: CP= 416±0.34, EE=11±0.2, NDF=273±1.04, ADF=194±2.15 and Ash= 80±0.26. Degradable coefficients, ruminal and post-ruminal disappearance of DM and CP are summarized in Table 1.

**Table 1** *In situ* degradability coefficients and rumen and post-rumen disappearance (Mean with SEM for 10 samples) of DM and CP of rapeseed meal (SLM sp.)

| Item | DM | CP |
|---|---|---|
| Rapidly degradable fraction | 0.32±0.02 | 0.33±0.02 |
| Slowly degradable fraction | 0.49±0.02 | 0.58±0.02 |
| Fractional degradation rate | 0.09±0.01 | 0.12±0.01 |
| Ruminal disappearance | 0.68±.0.03 | 0.71±0.04 |
| Post-ruminal disappearance of ruminal undegradable | 0.52±0.04 | 0.66±0.05 |
| Total tract disappearance | 0.83±0.03 | 0.91±0.04 |

**Conclusions** The crude protein of rapeseed meal, evaluated in the present study, was almost high and comparable with other protein source like soybean meal. Rapidly and slowly degradable fractions of RSM for DM and CP indicated that more than 0.85 of this feed could be digested in the rumen. Total tract DM and CP disappearance of RSM measured by mobile bag was the same as the data reported for soybean meal by Danesh Mesgaran and Stern (2005). The data showed that this variety of RSM has a good ruminal and post-ruminal digestibility.

**Acknowledgments** Financial support of Excellence Centre for Animal Science is gratefully acknowledged.

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# Chemical composition, dry matter and protein degradability coefficients of pistachio hulls silage treated with urea and molasses

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**Introduction** Pistachio hulls are pistachio processing by-products produced during de-hulling of pistachio nuts soon after harvesting. Total pistachio by-products in Iran have been increased at an average rate of about 300000 Tons per year. Chemical composition of Pistachio hulls contained 12%CP, 5%EE, 34% NDF, 21% ADF, and 9% ash (DM basis). The dry matter content of fresh Pistachio hulls is about 28%. Reducing the moisture content of this by-product with dryer systems is a cost effective process and can not be applied by most of the producers. Finding other alternative methods for preserving and using this by-product is highly required. The objective of the present study was to assess changes in Pistachio hulls silage (PHS) quality associated with the inclusion of different levels of urea (U) and molasses (M), and to compare *in situ* DM and protein degradability of PHS with dried pistachio hulls (DPH).

**Materials and methods** Pistachio hulls were ensiled with urea (0.0 or 0.15 % of DM) and beet molasses (0.0, 1.5, 3, and 4.5% of DM) in a completely randomized design with factorial arrangement of 2×4. Treatments were pistachio hulls ensiled with 0% U + 0% M ($T_1$), 0% U + 1.5% M ($T_2$), 0% U + 3% M ($T_3$), 0% U + 4.5% M ($T_4$), 0.15% U + 0% M ($T_5$), 0.15% U + 1.5% M ($T_6$), 0.15% U + 3% M ($T_7$), and 0.15% U + 4.5% M ($T_8$). Treatments were preformed in small laboratory silos (3 replicas for each treatment) for 35 days. Silage DM was determined using Air-forced oven (60°C, 48 h) and the chemical composition of samples was determined using the standard procedures of AOAC (1990). PH was determined in silage extract. Data were analyzed using the GLM procedure of SAS. DM and protein degradability of the dried and ensiled pistachio hulls were measured by *in situ* technique using two fistulated Holstein steers (400±12 kg). The animals fed a 40:60 concentrate: forage diet. The samples were milled (2 mm screen) and weighed (5 g DM) into bags (12×19 cm) made of polyester cloth with 52µm pore size (8 bags per each sample). The bags were incubated in the rumen for 0, 2, 4, 8, 16, 24, 48, 72, and 96 h. The degradable coefficients of DM and CP were determined using the equation of $P=a + b (1 – e^{-ct})$.

**Result** The obtained results are summarized in Tables 1 and 2. Addition of molasses to silages led to significantly ($P<0.05$) higher dry matter content. The addition of urea to the silage increased its CP content significantly ($P<0.05$). Both urea and molasses showed a significant effect on pH and N-$NH_3$ ($P<0.05$). Fractional degradation rate constant for DM and CP was not influenced by the treatments (Table2).

**Table 1** Chemical composition of pistachio hull silage treated with urea(U) and molasses(M)

| | Treatment | | | | | | | | Effects | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Item | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ | $T_6$ | $T_7$ | $T_8$ | SEM | U | M | U×M |
| DM(g/kg) | 276 | 296 | 289 | 307 | 288 | 308 | 302 | 310 | 12.9 | ns | * | ns |
| pH | 5.27 | 4.52 | 4.54 | 4.63 | 6.62 | 4.73 | 4.63 | 4.77 | 0.71 | * | * | * |
| NDF(g/kg) | 336 | 345 | 347 | 344 | 334 | 348 | 337 | 337 | 7.5 | ns | ns | ns |
| ADF(g/kg) | 211 | 204 | 211 | 206 | 207 | 207 | 203 | 204 | 4.8 | ns | ns | ns |
| CP (g/kg) | 118 | 118 | 117 | 117 | 140 | 145 | 141 | 148 | 11.4 | * | ns | ns |
| N-$NH_3$ | 6.28 | 5.88 | 5.87 | 5.59 | 14.2 | 9.21 | 8.04 | 8.97 | 3.78 | * | * | * |

*: $P< 0.05$; ns: $P>0.05$

**Table 2** *In situ* DM and protein (mean ± SE) rumen degradation coefficients (a, b, c) of dried pistachio hulls (DPH), pistachio hull silage(PHS) and pistachio hull silage treated with 0.15%urea+1.5%molasses(PHS+U,M).

| Treatment | DM | | | CP | | |
|---|---|---|---|---|---|---|
| | a | b | c | a | b | c |
| DPH | 0.439±0.007 | 0.421±0.017 | 0.025±0.03 | 0.554±0.012 | 0.418±0.068 | 0.016±0.005 |
| PHS | 0.379±0.006 | 0.458±0.020 | 0.022±0.02 | 0.451±0.010 | 0.435±0.038 | 0.020±0.004 |
| PHS+U,M | 0.465±0.007 | 0.427±0.025 | 0.020±0.03 | 0.660±0.019 | 0.335±0.125 | 0.015±0.011 |

*a:* rapidly degradable fraction, *b:* slowly degradable fraction, *c:* fractional degradation rate constant ($h^{-1}$)

**Conclusions** There were interactions between urea and molasses in the effect on pH and N-$NH_3$. Urea and molasses had opposite effects on the pH and N-$NH_3$ content of silages. Low pH in molasses treated silages may be resulted from the fermentable carbohydrates of molasses. Low concentration of N-$NH_3$ in pistachio hulls silage treated with molasses can be related to slow proteolysis. Rapidly degradable fraction of DM and CP of silages treated with urea and molasses may be related to the increase of soluble N and organic matter. The result of the present study indicated that ensiling could be employed as a simple method for preserving and improving the Pistachio hulls as a ruminant feedstuff.

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# Effect of heat processing on ruminal and post-ruminal disappearance of essential and non-essential amino acids of Iranian whole soybeans

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**Introduction** Whole soybean (SB) is used as a high energy-protein supplement for dairy cows, but the protein is highly degradable by rumen microbes. Various chemical and physical processing has been suggested to decrease ruminal protein degradability, that heat processing is the most commonly used physical method. Modern systems for protein evaluations in ruminants are moving in the direction of predicting absorption of amino acids from the small intestine, so the determination of intestinal digestibility of amino acids is of special importance particularly in heat-treated feedstuffs. The objective of this research was to elucidate the effects of roasting and steep-roasting on ruminal and post-ruminal disappearance of essential and non-essential amino acids (EAA and NEAA) of Iranian SB.

**Materials and methods** In this study two Iranian cultivars of soybeans (Sahar and Williams) were used. The seeds were roasted using a drum roaster with an exit temperature of 140 to 145 °C. Then, some seeds were gradually cooled (about 1 h) and the rest were immediately placed in isolated barrels, without cooling, and covered with canvas for about 45 min (steeping) and then cooled. Essential AA and NEAA ruminal and post-ruminal disappearance of feeds were evaluated using the *in situ* mobile bag technique with two ruminally fistulated (430 ± 10 kg) and two intestinally cannulated (400 ± 8 kg) Holstein steers (2 bags per sample was put in each steer). The samples were dried using a forced-air oven at 96 °C for 48 h, ground to pass through a 2-mm screen, and then analyzed for AA content. Amino acid analysis of feeds and residues of ruminal degradation and post-ruminal digestion was performed by cation-exchange chromatography using a Beckman System Gold Amino Acid analyzer with post-column ninhydrin detection. Ruminal disappearance of AA was determined by incubating the feed samples in the rumen for 12 h and calculations as described by Subuh et al. (1996) were used for determining the post-ruminal disappearance of EAA and NEAA. Experiments were designed as completely randomized with a factorial arrangement of treatments and the data were analyzed using the GLM procedure of SAS (SAS, 1999), with the factors in the model consisting of heat processing, soybean cultivars and their interaction. The main effects of factors and their interaction were compared using Duncan test.

**Results** Heat processing reduced ruminal disappearance of EAA and NEAA of processed SB significantly (P<0.001), while roasting increased the post-ruminal disappearance of EAA and NEAA significantly (P<0.001) and steeping intensified it (Table 1), showing beneficial effects of steeping beyond roasting.

**Table 1** Effect of heat processing[a] and cultivar (Sahar, S; Williams, W) on proportion of ruminal and post-ruminal disappearance of feed EAA and NEAA

| | P[b] | | | | C | | | P × C |
|---|---|---|---|---|---|---|---|---|
| | NOP | ROS | STR | SEM | S | W | SEM | SEM |
| Ruminal | | | | | | | | |
| EAA | 0.56 x | 0.24 y | 0.25 y | 0.015 | 0.34 | 0.36 | 0.008 | 0.015 |
| NEAA | 0.57 x | 0.25 y | 0.24 y | 0.011 | 0.40 | 0.36 | 0.007 | 0.013 |
| Post-ruminal | | | | | | | | |
| EAA | 0.67 z | 0.77 y | 0.83 x | 0.012 | 0.75 | 0.77 | 0.009 | 0.016 |
| NEAA | 0.65 z | 0.74 y | 0.80 x | 0.011 | 0.71 | 0.75 | 0.009 | 0.015 |

[a] NOP: non-processing; ROS: roasting; STR: steep-roasting.
[b] P: soybean processing; C: cultivar; P × C: interaction of processing and cultivar.
Means in the same row with no common letters differ (P<0.001).

**Conclusions** Heat processing (roasting at 140 to 145 °C and roasting plus steeping for 45 minutes) of SB reduced ruminal degradation and increased post-ruminal disappearance of EAA and NEAA, perhaps due to the destruction of trypsin inhibitor. Consequently, these processing methods seem to shift the site of AA digestion from the rumen to the small intestine and increase the amount of undegraded AA digested in small intestine. Steeping improved the post-ruminal digestibility of EAA and NEAA beyond the effects of roasting. There was no significant difference between the two Iranian soybean cultivars in relation to AA ruminal degradability and post-ruminal disappearance.

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## *In vitro* digestion and ruminal degradation of soybean hulls

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**Introduction** Dairy producers use soyhulls, a byproduct of soybean processing, to replace either grain or forage in diets of lactating dairy cows. In view of the nutritional and economical value of soyhulls it is anticipated that this practice will continue to increase in popularity among nutritionists and producers of ruminant animals. According to the NRC (2001), SH contain 60.3% NDF and 44.6% ADF on a DM basis. Also The CP content of SH averaged 11.8%, which is within the range of 13.9± 4.6%. The objectives of this paper are to evaluate the *in vitro* DM and OM digestibility and *in situ* degradability of DM, CP and NDF contents of soybean hulls.

**Materials and methods** 13 samples of soybean hulls collected and duplicated of each SH sample were ruminally incubated in Dacron bags in four Taleshi steers (550 ± 40 kg body weight) to determine the rates of DM, CP and NDF disappearance. The steers had fed at Maintenance level with a diet that had 60:40 hay to concentrate ratio at 0800 and 1500 h. Samples of SH were dried in an oven at 60°C for 48 h, ground (2-mm screen), and weighed (approximately 5.0 g) into 10-cm x 20-cm Dacron bags with a pore size of 50 µm. The bags were tied shut and ruminally incubated according to the method of Nocek and Russell (1988). The duplicated bags were incubated for 0, 4, 8, 16, 24, 48, 72 and 96 h and were placed in the rumen in reverse sequence (i.e., 24-h bags first). Upon removal, the bags were rinsed with cool running tap water for approximately 15 min and dried in an oven at 60°C for 48 h. Dry bags and residues were weighed, and DM disappearance was determined by correcting for contamination of the blank of the corresponding incubation time. Samples of feedstuffs and residues were analyzed for NDF concentration (Van Soest et al., 1991), and rates of DM, OM, CP and NDF disappearance were calculated using a nonlinear procedure (Orskov and McDonald, 1979). Just before feeding, 500 ml of ruminal fluid were withdrawn from each steers and strained through four layers of 60 mesh cheesecloth. Ruminal fluid from each steers was used to inoculate triplicate tubes (120 x 26 mm plastic) containing 0.5 g of the appropriate SH ground through a 2-mm screen and four blank tubes. A 4:1 ratio of McDougall's buffer and ruminal fluid was used. Tubes were flushed with *CO2,* capped and incubated (39°C) in a water bath for 48 h, then removed and frozen at -40°C. Subsequently, tubes were thawed and centrifuged at 2,500 x g for 15 min. The supernatant fluid was removed and the pellet was resuspended in a 1 *N* HCI-pepsin solution and reincubated (39'C) for 48 h. Remaining fluid and feed residue was filtered through modified Buchner funnel with ashless filter paper3. Digestibility was calculated by standard procedures, accounting for blank tube correction.

**Results** *In vitro* digestibilities of DM and OM for soybean hulls were 79.8 and 87.2% respectively. These results show that soybean hulls have high potenrial of digestion. Degradibility of DM, CP and NDF and potential of its digestion are given in table 1 and 2. Also rate of degradation are given. As you seen soybean hulls have high potential of degradation for DM, CP and NDF. In hour 24 you can see that 70 % of DM, CP and NDF have been degraded.

**Table 1 and 2** *In vitro* digestion and ruminal degradation of soybean hulls

| Incubation hours | Degradability (%) | | |
|---|---|---|---|
| | DM | NDF | CP |
| 0 | 17.54 | 5.51 | 29.35 |
| 4 | 22.09 | 7.89 | 37.84 |
| 8 | 29.09 | 17.86 | 44.65 |
| 16 | 55.85 | 45.07 | 65.53 |
| 24 | 73.15 | 67.13 | 70.57 |
| 48 | 90.79 | 92.35 | 84.92 |
| 72 | 93.59 | 95.07 | 87.71 |
| 96 | 93.64 | 95.87 | 88.15 |

| Potential of degradability and rate of digestion | | | |
|---|---|---|---|
| Parameter | DM | NDF | CP |
| a (%) | 17.54 | 5.51 | 29.35 |
| b (%) | 77.95 | 93.23 | 59.30 |
| c (%/h) | 5.67 | 5.11 | 5.76 |

| Passage rate (%/h) | Effective degradability (%) | | |
|---|---|---|---|
| | DM | NDF | CP |
| 1 | 81.4 | 81.2 | 79 |
| 2 | 71.1 | 69.7 | 71.9 |
| 5 | 52 | 43.4 | 58.5 |

**Conclusion** The results show that DM, NDF and CP content of SH degraded high amounts and in high rates. High digestibility of soybean hulls and high nutritional value of its shows that it can supply high energy and intermediate protein for animals. Then it can be used in replacement of concentrate especially in replace of grains (for example in replacement of corn grain that have high energy and low protein content).

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# The effect of different concentrate: Lucerne hay ratio on crude protein degradation of various feeds using *in situ* technique

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**Introduction** Available information on the effect of ruminal pH on degradation of crude protein of various feeds is scarce. It has been reported that in a high concentrate diets, protein degradation is usually reduced (Molero *et al*., 2004). This reduction has been attributed to lower ruminal pH, which causes the changes in protein solubility (Loerch *et al*., 1983) and reduces fibrolytic activity of rumen microflora. There is also limited information concerning the requirements for rumen degradable N (RDN) when high-grain diets are given to steers, and most of that information has been obtained using urea as the source of RDN. The aim of the present experiment was to evaluate the effect of different concentrate to lucerne hay ratios (60:40, 70:30, 80:20 or 90:10) on ruminal crude protein degradation kinetic of lucerne hay, barley grain, soybean meal and fish meal.

**Materials and methods** The experimental feeds were lucerne hay, barley grain, soybean meal and fish meal. Samples were dried using a forced-air oven at 96 ºC for 48 h. All feed samples were ground to pass through a 2-mm screen, then, analyzed for total N (Kjeldahl method). The samples were incubated in the rumen of four Holstein steers (300±15 kg body weight) with ruminal fistulae. Animals were fed 6.8 kg of DM of diets (2 times per day) differing in concentrate (155 g CP $kg^{-1}$ of DM; 30% maize, 34% barley, 8% soybean meal, 5% sugar beet pulp, 10% wheat bran, 12% cottonseed meal, 0.3% CaCo3, 0.5% mineral and vitamin premix, 0.2% salt) to lucerne hay ratios as 60:40 ($C_{60}$:$L_{40}$), 70:30 ($C_{70}$:$L_{30}$), 80:20 ($C_{80}$:$L_{20}$) and 90:10 ($C_{90}$:$L_{10}$) in a 4X4 Latin square design (28 days of each period). Samples (5 g DM) were placed in artificial silk bags (10×20 cm, 50 µm pore size) and incubated in the rumen for 0.0, 2, 4, 8, 16, 24, 48, 72 and 96 h (n=8) in each period. In addition, the pH of the ruminal fluid samples was measured with a portable pH meter before the morning feeding to 8 hours post feeding (interval 15 min) at days of 24 to 28 on each period. Data of CP degradation beyond the lag-time were further adjusted to a negative exponential model [$P= a+b(1-e^{-ct})$, where P= fraction degraded in the time t, a= rapidly degradable fraction, b= slowly degradable fraction, c= fractional degradation rate and t= incubation time]. Effective degradability of CP (out flow rate= 0.08/h) of the feed samples was also calculated. All data were subjected to least squares ANOVA using the GLM procedure of SAS at $p< 0.05$.

**Results** Minimum daily ruminal pH decreased from 6.40 ($C_{60}$:$L_{40}$) to 5.34 ($C_{90}$:$L_{10}$). The results showed that the CP degradation parameters of the feeds are influenced by the diet composition (Table 1).

**Table 1** *In situ* CP degradation parameters (Mean±SE) of various feeds determined in steers fed diets differing in concentrate to lucerne hay ratios

| Feeds | Parameter* | Concentrate:lucerne hay ratio† | | | | SEM |
|---|---|---|---|---|---|---|
| | | 60:40 | 70:30 | 80:20 | 90:10 | |
| Lucerne hay | a | 0.39±0.01 | 0.38±0.01 | 0.40±0.01 | 0.40±0.01 | 0.01 |
| | b | 0.44±0.02 | 0.49±0.01 | 0.41±0.01 | 0.45±0.01 | 0.01 |
| | c | 0.07±0.01 | 0.06±0.01 | 0.08±0.01 | 0.06±0.01 | 0.01 |
| Barley grain | a | 0.21±0.04 | 0.25±0.03 | 0.30±0.03 | 0.21±0.05 | 0.04 |
| | b | 0.61±0.04 | 0.57±0.03 | 0.55±0.04 | 0.61±0.05 | 0.03 |
| | c | 0.14±0.03 | 0.23±0.03 | 0.12±0.02 | 0.17±0.04 | 0.03 |
| Soybean meal | a | 0.05±0.02 | 0.06±0.02 | 0.05±0.02 | 0.07±0.05 | 0.03 |
| | b | 0.62±0.15 | 0.89±0.16 | 0.72±0.13 | 0.56±0.08 | 0.09 |
| | c | 0.02±0.01 | 0.01±0.01 | 0.02±0.01 | 0.04±0.02 | 0.01 |
| Fish meal | a | 0.19±0.01 | 0.19±0.01 | 0.18±0.01 | 0.17±0.01 | 0.01 |
| | b | 0.51±0.05 | 0.47±0.03 | 0.44±0.02 | 0.43±0.02 | 0.02 |
| | c | 0.03±0.01 | 0.03±0.01 | 0.04±0.01 | 0.04±0.01 | 0.01 |

*: a= rapidly degradable fraction, b= slowly degradable fraction, c= fractional degradation rate; †: When the difference between means is greater than two times the SEM, it is considered as significant ($P < 0.05$).

**Conclusions** In the present study, diet composition significantly affected the degradation parameters, when ruminal CP degradation of the various feed samples were considered. Therefore, the extent of ruminal CP degradation is a function of rumen pH. The effect being more pronounced on the fractional degradation rate (*c*), when barley grain was evaluated. This parameter was stimulated by $C_{70}$:$L_{30}$, but, decreased when the animals fed $C_{80}$:$L_{20}$. In general, effective degradability of CP of the feed samples was decreased when animals fed $C_{90}$:$L_{10}$.

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# Effects of gamma irradiation on protein degradation of safflower meal in the rumen

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**Introduction** Safflower meal proteins are extensively degraded in the rumen. Attempts to decrease the degradability of feedstuffs proteins have involved treatment with heat, formaldehyde, tannic acid, acetic acid, xylose, and microwave (Sadeghi *et al.*, 2006). To our knowledge, no information is available concerning effects of gamma irradiation on ruminal protein degradation and type of safflower meal proteins that leave the rumen undegraded. The objectives of this study were to investigate effects of gamma irradiation on ruminal degradability and intestinal digestibility of safflower meal crude protein, and to monitor fate of true proteins of safflower meal in the rumen by using SDS-PAGE methodology.

**Materials and methods** The safflower meal (SM) samples were obtained from Jahan oilseed manufactory located 40 km west of Tehran (Iran). Safflower meal used in this study contained 301, 410, 305 and 80 g/kg DM crude protein, NDF, ADF and ash, respectively. Three SM samples (600 g each) were irradiated in gamma cell (Co-60) at doses of 25, 50 and 75 kGy. The procedure for incubation of nylon bags (two bags × 6 incubation times) in the rumen was the same as that described by Shawrang et al. (2007). Cows were fed 15 kg dry matter; a total mixed ration containing 700 g/kg of DM of high quality alfalfa hay and 300 g/kg of DM concentrate (171 g CP/kg of DM) twice daily at 08:00 and 16:00 h. Digestibility of CP was measured using mobile nylon bag technique (8 bags for each treatment). The protein subunits were fractionated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Chemical analyses were completed according to Association of Official Analytical Chemists (AOAC, 1995). Degradation kinetics was calculated using the model of Ørskov and McDonald (1979). Data were analyzed as a randomized complete block design, using animals as blocks using the GLM procedure of SAS (1996).

**Results** There were differences ($P$<0.05) for DM and CP degradation characteristics and effective degradability values between untreated and gamma irradiated SM. Gamma irradiation decreased immediately soluble fraction and degradation rate and increased potentially degradable fractions of DM and CP.

**Table 1** The crude protein degradation kinetics of untreated and gamma irradiated safflower meal(SM)

| | Washout fraction | Potentially degradable fraction | Degradation rate (per h) | ED† 0.02 | ED 0.05 | ED 0.08 |
|---|---|---|---|---|---|---|
| Untreated SM | 0.456[a] | 0.469[d] | 0.105[a] | 0.850[a] | 0.774[a] | 0.722[a] |
| 25 kGy g-irradiated SM | 0.325[b] | 0.520[c] | 0.066[b] | 0.724[b] | 0.620[b] | 0.560[b] |
| 50 kGy g-irradiated SM | 0.285[c] | 0.538[b] | 0.063[b] | 0.693[c] | 0.585[c] | 0.522[c] |
| 75 kGy g-irradiated SM | 0.258[d] | 0.553[a] | 0.063[b] | 0.678[d] | 0.566[d] | 0.502[d] |
| s.e. | 0.0112 | 0.0172 | 0.0052 | 0.0215 | 0.0182 | 0.0175 |

[a, b, c, d] Means in the same column with different superscripts differ ($P$<0.05).
† ED: Effective degradation calculated with a solid outflow rate from the rumen of 0.02, 0.05 and 0.08 per h.

From the slab gel analysis, SM proteins were composed of two major components A and B, accounting for approximately 34 and 51 percent of the total meal protein, respectively. Both proteins were multi-subunits. The molecular weights of 31.9, 26.0, 21.4, 19.5 kDa for A subunits and 8.0, 9.6 kDa for B subunits were observed in this study. Electrophoretic and densitometric analysis of untreated SM protein residues revealed that B subunits were degraded completely within 4-h, whereas the four subunits of A were not degraded after 12-h of incubation. In gamma irradiated SM, B subunits were resistant until 12-h incubation. The four subunits of A were more resistant to degradation. There were differences ($P$<0.05) between crude protein digestibility of untreated and gamma irradiated safflower meal. Crude protein digestibility of untreated, 25, 50 and 75 kGy gamma irradiated SM at 16-h of ruminal incubation period were 807, 821, 852 and 879 g/kg, respectively.

**Conclusions** The results obtained in this study indicate that the degradation characteristics of safflower meal proteins could be altered by gamma irradiation. Gamma irradiation of safflower meal caused cross-linking and aggregation of the polypeptide chains, resulting in decrease of effective degradation of crude protein in the rumen.

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Shawrang, P., Nikkhah, A., Zareh, A., Sadeghi, A.A., Raisali G. and Moradi, M. 2007. Effects of gamma irradiation on protein degradation of soybean meal in the rumen. *Animal Feed Science and Technology* (in press).

# *In situ* dry matter and crude protein degradation kinetics of sunflower meal

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**Introduction** The use of by-product in animal nutrition is necessary since it may increase the availability of feed for animal and avoid accumulation that contributes to environmental problems. Sunflower meal is a source of supplemental protein for livestock feeding. The amount of hull or fibre in sunflower meal is the major source of variation in nutrient content of this feed. Methionin concentration in sunflower meal is high compared with other protein sources such as cottonseed meal. Sunflower meal without hulls has high degradability than sunflower meal with hull. This study was conducted to evaluate the chemical composition and *in situ* dry matter (DM)and crude protein (CP) degradability of three different sources of sunflower meal (high, medium and low fat).

**Materials and methods** Three sources of sunflower meal with hulls were provided. Samples were 1- Sunflower meal containing 165 g/kg fat (SFM165), 2- Sunflower meal containing 125 g/kg fat (SFM125) and 3-Sunflower meal containing 25 g/kg fat (SFM25). Chemical composition (DM, CP, Ash and NDF) were determined using standard procedures (AOAC, 1990). Dry matter and crude protein degradability of the samples were measured by *in situ* technique using two fistulated Holstein steers (400±12 Kg, body weight). The animals fed a 40:60 concentrate: forage diet. The experimental samples were milled (2-mm screen) and weighed (5 g, DM) into bags (12x19 cm) made of polyester cloth with 52 µm pore size (8 replicates per each treatment). The bag were incubated in the rumen for 2, 4, 6, 8, 16, 24, 48, 72 and 96 h after being soaked in distilled water (38 °C) for 10 min. Bags also were washed with cold tap water to estimate the wash-out at zero time. After each incubation time, the removal bags were hand-washed with cold tap water, and then dried in a forced-air oven (60 °C, 48 h). The degradable parameters of DM and CP were determined using the equation of $P=a + b (1 - e^{-ct})$, where P: potential degradability, a: rapidly degradable fraction, b: slowly degradable fraction, c*:* fractional degradation rate constant ($h^{-1}$). Data of effective degradability of CP and DM (out flow rate=0.08 $h^{-1}$) were analyzed using GLM of SAS in a completely randomized design ($p<0.05$).

Results Chemical composition of the various sunflower meals is shown in Table1. Ruminal degradation parameter (a, b, c) of dry DM and CP are summarized in Table 2. Sunflower meal containing 25 g/kg fat had the most rapidly degradable fraction (a) and the least fractional degradation rate (c).

Table 1 Chemical composition of three different sources of sunflower meals

| Chemical composition | Sunflower meal [1] | | |
|---|---|---|---|
| | SFM165 | SFM125 | SFM25 |
| EE ( g/kg ) | 165 | 125 | 25 |
| CP ( g/kg ) | 282.1 | 259 | 338.2 |
| Ash ( g/kg ) | 50 | 53 | 84 |
| DM | 93.75 | 92.5 | 94 |

1- SFM165: Sunflower meal containing 165 g/kg fat; SFM 125: Sunflower meal containing 125 g/kg fat; SFM25: Sunflower meal containing 25 g/kg fat

Table 2 in *situ* DM and CP (mean ± SE) degradation parameters (a, b, c) of various sunflower meals

| item | DM | | | CP | | |
|---|---|---|---|---|---|---|
| | SFM165 | SFM125 | SFM25 | SFM165 | SFM125 | SFM25 |
| a | 0.41±0.03 | 0.44±0.03 | 0.45±0.03 | 0.48±0.03 | 0.64±0.03 | 0.66±0.02 |
| b | 0.27±0.03 | 0.28±0.03 | 0.26±0.03 | 0.48±0.01 | 0.31±0.03 | 0.29±0.02 |
| c | 0.21±0.05 | 0.19±0.05 | 0.12±0.03 | 0.47±0.01 | 0.27±0.06 | 0.10±0.02 |
| ED | 0.614 | 0.645 | 0.606 | 0.870 | 0.90 | 0.831 |

a: Rapidly degradable fraction; b*:* Slowly degradable fraction; c*:* Fractional degradation rate constant ($h^{-1}$)
ED: effective degradability (out flow rate= 0.08 $h^{-1}$)

**Conclusions** Results of the present experiment indicated that the rapidly degradable fraction (a) of DM and CP of SFM125 and SFM25 were higher than those of SFM165. While there was no difference between SFM125 and SFM25. Fractional degradation rate (c) of SFM25 was notably lower than the other samples when DM and CP degradation was considered. In General, results of the present experiment showed that the potential degradation of sunflower meal might be affected by the fat content of the samples.

**Acknowledgements** The authors wish to acknowledge for funding and technical supporting from Ferdowsi University of Mashhad and Centre of Excellence for Animal Science.

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# The effect of tannins in grape pomace on the protein fractions in soybean meal

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**Introduction** Soybean meal (SBM) is commonly used as protein supplement in the ration of high producing dairy cattle. It has a good palatability and acceptable amino acid balance. However, SBM has relatively low protein efficiency because of the extensive ruminal degradation. Recently, tannins have become a matter of interest in ruminant nutrition because of their potentially beneficial effects on decreasing the ruminal degradation of dietary protein (Barry and McNab, 1999). Several methods (*in vivo, in vitro, in situ* and solubility methods) have been proposed to characterize the dietary protein. Cornell Net Carbohydrate and Protein System (CNCPS) have a submodel in which crude protein is partitioned into five fractions with different degradation rates. Hence, the objective of this study was to determine the protein fractions of soybean meal treated with tannin in grape pomace.

**Materials and methods** Tannin treated SBM were prepared as follows. SBM (945 g/kg DM; 540 g crude protein/Kg DM; 116 g ash/kg DM) were milled and passed through a 2 mm mesh to get a finely ground mill. Purified tannins were diluted in aqueous solution (10% w/v) using the ultrasonic water bath to dissolve the CT completely in distilled water. The volume of 10% tannins in solution added to obtain 1.5, 3, 4.5 and 6% tannins. Crude protein (CP) was calculated as 6.25 times the N concentration, determined using the Kjeldahl method (AOAC, 1990, method 984.13). Non-protein nitrogen (A) fraction was determined following the procedure outlined by Licitra et al. (1996). The remaining $B_1$ (rapidly degradable), $B_2$ (intermediate degradable), $B_3$ (slowly degradable), and C (indigestible) fractions (Van Soest, 1994) were determined with the aid of neutral detergent (ND) and acid detergent (AD) solutions and the residues analyzed for N content. Triplicate samples for each treatment were used. All obtained data were subjected to one-way analysis of variance. The effect of treatments (different levels of CT) partitioned into linear and quadratic components by using orthogonal polynomials.

**Results** The different protein fractions of untreated SBM and tannin treated SBM are shown in Table 1. Fractions A and B2 declined with the rising level of tannin (linear and quadratic effects, $P<0.01$). However, B1 and B3 fractions increased in a dose-dependent manner when compared with control (0%) (Linear and Quadratic effects, $P<0.01$). Similarly, adding tannins to SBM increased C fraction which is unavailable for animal (linear effect, $P<0.01$).

**Table 1** Effect of different doses of condensed tannin on protein fractions (g/kg DM) in soybean meal protein fractions

| Percentage of CT | A | B1 | B2 | B3 | C | Total CP |
|---|---|---|---|---|---|---|
| 0 | 56.57 | 51.50 | 406.0 | 10.63 | 16.00 | 540.7 |
| 1.5 | 42.17 | 66.17 | 382.3 | 32.03 | 16.46 | 539.13 |
| 3 | 33.80 | 73.40 | 373.3 | 35.20 | 18.34 | 534.13 |
| 4.5 | 32.16 | 75.63 | 368.0 | 35.37 | 19.50 | 530.66 |
| 6 | 18.14 | 87.67 | 360.2 | 52.47 | 21.07 | 539.55 |
| SEM | 0.87 | 1.01 | 5.21 | 0.19 | 1.31 | 5.9 |
| P-values | | | | | | |
| Linear | ** | ** | ** | ** | ** | NS |
| Quadratic | ** | ** | ** | ** | NS | NS |

A: Non-protein nitrogen; B1: rapidly degradable fraction; B2: intermediate degradable fraction; B3: slowly degradable fraction; C: indigestible fraction; CP: crude protein; ** $P<0.01$. n=3

**Conclusions** The present experiment suggests tannins in grape pomace can decrease the Non-protein nitrogen fraction and increase B fractions which have more chance to pass into the intestine. However, increasing the fraction C should be considered as a negative point in terms of protein availability for animals.

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# The effects of prior handling experiences on the stress responses of semi-feral foals at auction

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**Introduction** Welfare is often defined as the state of an individual to cope with the environment in which it finds itself (Fraser and Broom, 1997). This statement is further endorsed by the assertion that stress is triggered by environmental stimuli that cause a defence reaction or 'stress response' (Mostl and Palme, 2002). Stress can be measured by alterations in behaviour and through changes in physiological parameters. In a recent report, the Farm Animal Welfare Committee (2005) postulated that elevated levels of stress in semi feral ponies at auction maybe apparent in those ponies herded through auction sale rings by handlers displaying limited competence and knowledge of equine husbandry. Aversive handling and the consequent fear that animals feel towards humans can be a major source of stress and should be considered a welfare concern (Rushen *et al.,* 1999). Research into aversive handling has a propensity to be more focused on intensively farmed production animals. However, it is apparent that the fast movement of pony stock through sale rings, and the often aggressive manner in which this can be carried out, may not be considered optimal for the welfare of these ponies. The aim of this study was to investigate the effects of prior handling experience on the stress response of semi feral foals at auction.

**Materials and methods** 74 registered New Forest (NF) ponies between the ages of 4 months and 2 years were randomly selected from a total of 179 animals. Horses were blocked for age and handling status. Those animals not broken to halter and therefore herded through the ring were classed as unhandled. Ponies initially led into the ring, and then turned loose were classed as handled. Ponies were videoed whilst in the ring, and an ethogram study was carried out to establish stress levels through analysis of exhibited behavioural parameters. Behavioural frequencies and durations were recorded and analysed for avoidance (trot/canter), flight, vocalisation, ears back, jump and investigation. A Mann Witney U test was used to test for an association between pony behaviour and handling status.

**Results** 16 of the 74 ponies were classified as handled; the remaining 58 being classed as unhandled and therefore herded into the ring. Unhandled foals showed significantly higher values for behaviours that could indicate an increased stress response. Percentage time spent in flight or displaying avoidance behaviour was found to be very highly significant ($U=149$, $P<0.001$). The mean frequency of both jumps performed ($U= 105.5$, $P<0.001$) and ears back ($U= 266.5$, $P<0.01$), were both found to be very highly significant showing clear differences between the handled and unhandled groups. Investigative behaviour was also found to be significantly higher for unhandled foals ($U=293.5$, $P<0.01$). Behaviours less associated with stress, walk and stand, were both found to be very highly significantly, ($U=118$, $P<0.001$), and significantly higher ($U=311$, $P<0.05$), respectively, for handled foals. Vocalisation was found to be not significant.

**Table 1** Mean behavioural responses of handled vs. unhandled foals at auction

| Behaviour | Handled horses | Unhandled horses | U value | s.e.d. | Sig. |
|---|---|---|---|---|---|
| % Time in Flight/Avoidance (sec) | 21.73[a] | 50.40[b] | 149.0 | 14.335 | *** |
| % Time in Walk (sec) | 46.87[a] | 19.59[b] | 118.0 | 13.640 | *** |
| % Time Standing (sec) | 28.27[a] | 20.73[b] | 311.0 | 3.770 | * |
| Vocalisation Frequency (freq.) | 0.438[a] | 1.017[a] | 349.0 | 0.289 | NS |
| Jump Frequency (freq.) | 0.125[a] | 3.034[b] | 105.5 | 1.455 | *** |
| Ears Back Frequency (freq.) | 7.688[a] | 11.10[b] | 266.5 | 1.706 | ** |
| Investigative Frequency (freq.) | 0.063[a] | 1.241[b] | 293.5 | 0.589 | ** |

[a, b]Mean values with differing superscripts are * Significant ($P<0.05$), ** Highly significant ($P<0.01$), *** Very highly significantly different ($P<0.001$)

**Conclusions** Unhandled ponies spent significantly more time ($P<0.001$) performing avoidance and jump behaviour, and ears back ($P<0.01$) than handled ponies. Avoidance/flight reactions are considered welfare indicators and the duration can be measured as an index of disturbance. The results are in agreement with previous research by Sondergaard and Halekoh (2003) who found that unhandled horses reacted more to humans and novelty and showed more avoidance/flight behaviour. Conversely, handled ponies spent significantly more time standing and walking, possibly indicating a reduced fear response due to prior conditioning. Investigative behaviour was significantly higher in unhandled foals; common in horses responding to novel stimuli and has been shown to be performed at a higher frequencies in unhandled horses in novel object tests (Lansade *et al.*, 2004). The results show that ears being back occurred significantly more with the unhandled ponies ($P<0.01$).It can therefore be concluded that prior handling of foals appears to significantly reduce the occurrence of behavioural parameters specifically linked to a heightened stress response.

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# Opioid circuitry and the aetiology of equine oral stereotypy

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**Introduction** Stereotypic behaviour is generally attributed to a dysfunction of the basal ganglia, although confusion exists as to whether altered function stems from the dorsal (Caudatus and putamen) or ventral regions (nucleus accumbens). In rodents, imbalanced basal ganglia opioid physiology leads to enhanced efferent neural transmission from only the dorsal striatum to cortex. However, in horses performing oral stereotypy (crib-biting), neural alterations in dopamine receptor density have been recorded in both dorsal and ventral striatal regions (McBride and Hemmings, 2005) suggesting that both projections may be altered in the stereotypy phenotype. Indeed, if stereotypies are considered to stem from highly motivated appetitive behaviours, it seems probable that ventral elements of the basal ganglia 'reward' circuitry should also be involved If this is the case, then stereotypy performance has potentially rewarding consequences and could thus be employed as a coping strategy. To further address the issue of dorsal versus ventral striatum involvement in the equine oral stereotypy phenotype, comparisons of opioid receptor physiology between crib-biters and control horses were performed

**Material and methods** Opioid receptor (mu and delta) densities were quantified post-mortem in the brains of stereotypy (crib-biting) (N=8) and control (N=8) horses already designated for slaughter. Homogenates of the following brain regions: caudatus, putamen, nucleus accumbens [NA], ventral tegmental area [VTA] and substantia nigra, were incubated with a 2um concentration of either [3H] DAMGO for mu, or [3H] naltrindole for delta receptors, along with 10 decreasing cocentrations of unlabelled competitor spanning a 10um to 10nm range. Binding values were then plotted against a logarithmic scale of unlabelled (cold) ligand in order to estimate receptor density (Bmax) and affinity (Kd). A students t-test was used to compare average values obtained for the afore mentioned measurements of Bmax and Kd.

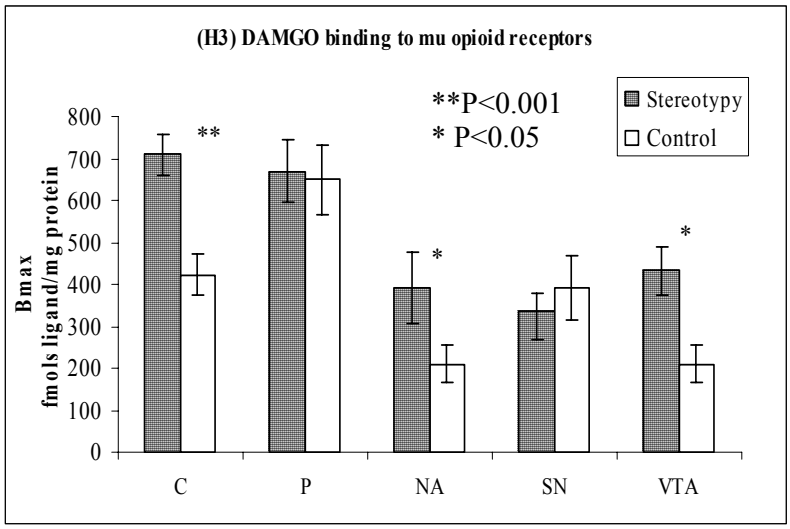


**Figure 1** Mean receptor density (Bmax (±SEM) in stereotypy horses versus control in the Caudatus (C ), Putamen (P), Nucleus Accumbens (NA), Substantia Nigra (SN), and Ventral Tegmental Area (VTA).

**Results** Binding data revealed that stereotypy horses had significantly higher densities (mean Bmax values [fmols bound ligand / mg] (±SEM) of mu receptors in the VTA (430.85 Vs. 217.47; t=2.13; P<0.05), NA (392.69 Vs. 211.43; t=2.08; P<0.05) and caudatus (709.50 Vs. 423.48; t=3.82; P<0.001) regions compared to controls. No significant results were recorded for delta receptor densities nor binding affinity (Kd) values for either opioid receptor subtype (see figure 1) .

**Conclusions** The increase in mu binding to dorsal regions such as the caudatus supports the idea of altered dorsal output via the direct pathway from striatum to thalamus. However, the increased mu opioid binding to ventral regions (NA and VTA) is indicative of facilitated ventral transmission (dopamine), arising from hyperpolarisation of inhibitory GABAergic interneurons of the VTA mediated by the mu receptor. Thus, both dorsal and ventral basal ganglia components show altered opioid physiology in the crib-biting animal. Ventral involvement implies that stereotypy activates pleasure circuitry, and could thus be employed as a coping strategy in times of stress. The common act of physical prevention could therefore prevent activation of the coping mechanism, leaving the animal prone to the deleterious effects of stress.

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# The effects of studs on forelimb kinematics during trot gait of the horse

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**Introduction** Studs are attached to horse shoes as anti slip devices for many performance horses (Dyson, 2000), with the aim of reducing the distance the hoof slides between impact and coming to rest. These anti slip devices are needed to prevent injury to the horse caused by over stretching of the joints and soft tissues during the slip or by loss of balance. Despite the practical benefits perceived by horse riders, it has been suggested that studs may have detrimental effects on the horse's kinematics and musculoskeletal system, possibly increasing jarring (Wilson *et al.,* 2001). The aim of this study was to measure the effects studs have on trot kinematics.

**Materials and methods** Eight event horses were filmed (at 50 Hz) trotting along a straight line on a grass surface with and without studs. Each horse was ridden by its regular rider. Three cameras were used to record the movement from the frontal and perpendicular views (with close up focus for hoof parameters and wide focus for stride length). Five repetitions were analysed using two-dimensional gait analysis (Equinalysis$^{TM}$) to measure stride length, maximal fetlock joint angle, over tracking distance (the horizontal distance between the placement of the hind hoof and front hoof), lateral movement of limb flight and time to the start of break over (when the heels leave the ground and rotate around the toe). Horses were assessed on a range of different ground conditions, due to environmental differences between data collection days. Ground conditions were assessed and categorised as very soft, soft or medium. Standing fetlock angle and hoof shape (heel to toe ratio) were also measured. Minitab Version 14 was used to assess differences between kinematics with and without studs, and the influence of ground conditions and fetlock / hoof conformation using General Linear Model.

**Results** Overall, there were differences between the stud / non-stud groups and an impact of ground conditions. Very soft ground induced a significantly higher ($p<0.01$, fig. 1) time to breakover when studs were used compared to times without studs. The other ground conditions with studs produced no significant changes in breakover time (fig. 1). Maximal fetlock angle also showed some differences. Very soft ground produced significantly lower maximal fetlock angles irrespective of the presence / absence of studs ($p < 0.05$, fig. 2). Fetlock angles on soft and medium ground showed evidence of changes with the introduction of studs (fig. 2).

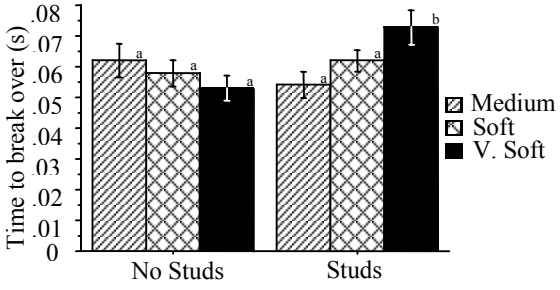


**Figure 1** Mean (±SE) time to breakover (seconds) with and without stud on different ground conditions (n=8). Letters denote heterogeneity between breakover times at the P<0.05 level.

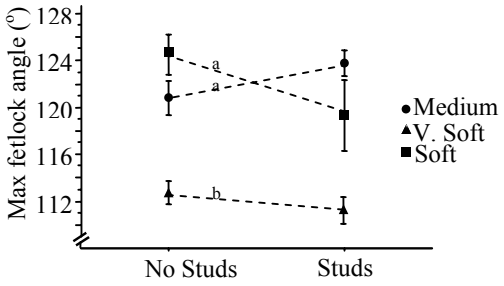


**Figure 2** Maximum fetlock angle recorded with and without studs on different ground conditions (n=8). Letters denote heterogeneity between ground types at the P<0.05 level.

**Conclusion** The addition of studs had an effect of delaying breakover on softer ground; however there was a minimal impact on fetlock angles. This suggests some alteration in the loading pattern of the hoof and may suggest potential risk of stress to the horse's musculoskeletal system. The study does however highlight the impact of ground conditions on kinematics and the potential "masking effect" this may have on any difference induced by the studs. Increasing sample size and refining surface classification may reveal the differences produced by studs use and would therefore be potential further work. Implications of this study are that movement may be affected by many exogenous factors and these need to be carefully considered to maximise performance.

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# An investigation of the total and airborne dust dynamics on two equine arena surfaces

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**Introduction** Respiratory disease poses a significant threat to the health and athletic performance of both horses and riders, necessitating the reduction of potential pollutants within equine environments (Deaton and Marlin, 2004). Enhanced management protocols for minimising respiratory irritants within equine housing have been developed, however knowledge of such pollutants in exercise areas remains ambiguous. The aim of this project was to investigate total dust levels and airborne surface dust dynamics during riding sessions on two different widely used arena surfaces.

**Material and methods** Two arenas (on the same site) were investigated. Arena 1 contained a relatively new surface (<5 years old) comprising high grade silica sand with elastic and polyester fibres; Arena 2 surface consisted of sand, dirt, organic matter and rubber chips (>10 years old). The arena surfaces were subjected to different management protocols, for example levelling and watering of the surfaces, according to the centre's usual routine. Dust measurements (n=10) were taken every day for four days during routine riding school lessons (duration was 45 minutes per session) where a consistent number (n=8) and type of horse were used. Total dust (the amount of dust collected during each session) was measured using an Apex personal sampler, which also recorded ambient temperature ($^{o}$C) at the start and end of each session. Airborne dust dynamics were measured using an infrared sensor (Microdust 880) by recording dust present in the air at one minute intervals throughout the session. Interactions between the variables were assessed using a General Linear Model.

**Results** Significantly greater amounts of total dust was collected from Arena 2 ($p<0.001$); total values also varied with trial day ($p<0.001$) (figure 1). Dust activity was significantly greater on Arena 2 ($p<0.05$) (table 1). Dust activity also varied with time of day ($p<0.001$), with most activity recorded in the morning. Temperature had no effect on the dust collected ($p>0.05$).

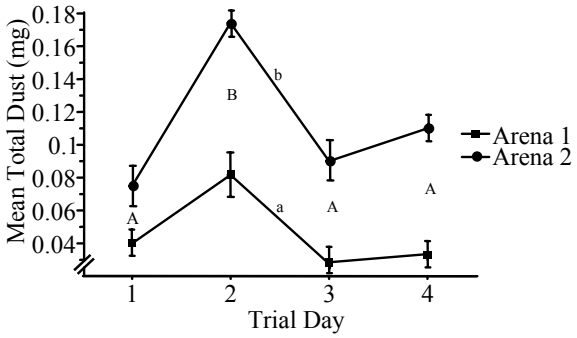


**Figure 1** Mean total dust (±1SE) (mg) from each arena during the four trial days, lower case letters denote heterogeneity between arenas, upper case letters denote heterogeneity between trial days; both at the P<0.05 level

**Table 1:** Microdust measurements from four trial days on two different arena surfaces showing minimum, maximum and mean average (± 1SE)

| Arena | Trial Day | Min dust activity (mg/m$^3$) | Max dust activity (mg/m$^3$) | Mean ±(SE) dust activity (mg/m$^3$) |
|---|---|---|---|---|
| 1 | 1 | 0.041 | 1.139 | 0.059 ±(1.06) |
| 1 | 2 | 0.051 | 1.561 | 0.070 ±(0.03) |
| 2 | 3 | 0.103 | 2.280 | 0.324 ±(4.06) |
| 2 | 4 | 0.124 | 1.590 | 0.770 ±(1.60) |

**Conclusion** Significant differences between the total dust levels and dust activity on the two arenas indicate that surface material and age have a strong influence on dust. The newer surface in Arena 1 produced lower total dust levels and lower dust activity rates. This surface was specifically designed for equestrian use, but was also subjected to greater management controls (watering and levelling). Significant variation between trial days supports the theory that dust is affected by daily environmental changes even though ambient temperature did not have a significant effect. The results emphasise the need for dust prevention measures, particularly on older surfaces and highlights the impact environmental influences have on dust levels. Examination of the differing compositions of each surface and the effect of specific environmental changes provide potential for future research.

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# Multiple forages as a behavioural enrichment for individually stabled horses

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## Introduction

Many domestic horses are kept in an environment very different from that of free-living horses, consuming a varied ad libitum forage based diet for up to 18 hours of the day (Harris, 1999). Cuddeford (1999) suggested that stabled horses may spend as little as 7 hours eating in a 24 hour period, with an increase in the time spent standing. Encouraging foraging behaviour, defined by Goodwin *et al.* (2002) to include sniffing, manipulating, biting, chewing or ingesting food, is thought to allow domesticated horses to spend more time eating, approaching the time spent on this activity in free-living horses. The diet of the free-living horse includes a selection of grasses and herbs (Putman *et al.*, 1987) whereas most domestic horses are provided with a single forage diet (Goodwin *et al.*, 2002). In a short term trial Goodwin *et al.* (2002) found that offering more than one source of forage to stabled horses resulted in them spending significantly more time foraging compared to a horse on a single forage diet. This effect was found to continue for longer periods by Thorne *et al.* (2005), however there appears to be no current literature on how many forages to feed within a multiple forage diet. The present study aimed to establish how many different forages to include in a multiple forage diet to maximise the time spent in foraging behaviour and minimise standing behaviour.

## Materials and methods

Six individually housed horses (aged 10 - 20 years, various breeds), acting as their own controls were fed six forage trial diets. Each trial was conducted over two days and a 6x6 Latin Square designed trial was chosen to minimise persistent effects from previous diets. The amounts of forage fed were based on intakes observed when ad libitum hay was available during the week prior to the forage enrichment trials and considerations of energy intake. Trial horses had all forages introduced to their diets prior to the start of data collection to allow microbial populations in the large intestine to adjust and minimise digestive upsets. Diet A was hay, diet B was diet A and mixed grass haylage, diet C was B and ryegrass haylage, diet D was C and mixed forage chaff, diet E was D and alfalfa chaff and diet F was E and un-molassed sugar beet pulp. The ratio of amount fed (kg) was 3 hay: 3 mixed grass haylage: 3 ryegrass haylage: 2 mixed forage chaff: 2 alfalfa chaff: 5 un-molassed sugar beet (soaked). Behavioural observations were taken using two methods: method one was point interval sampling every minute for 30 minutes after the introduction of trial diets and method two was point interval sampling every five minutes for hour periods at 1h, 3h and 5h after introduction. Water was provided on an ad libitum basis throughout the period of data collection. Wilcoxon's test for matched pairs was used to compare pairs of diets and forage types, and Friedman's rank test for K correlated samples was used to compare the different forage types.

## Results

Horses spent the most time eating on trial diet D (four forages), which was significantly more than on trial diet A, single forage, ($Z=-2.201$, $p=0.028$). Standing behaviour was least on trial diet D, significantly less than on trial diet A ($Z=-2.201$, $p=0.028$). No other multiples of trial diets were found to exhibit significantly differences in behaviours observed. The duration of mean eating time was found to be highly significantly different ($F= 19.976$, $df=5$, $p=0.001$) between forage types with horses spending significantly more time eating ryegrass haylage than all other diets ($Z$ = various, $p<0.001$).

## Conclusion

Forage enrichment by feeding multiple forages was found to significantly increase eating observations and significantly decrease standing observations only when four forages were fed (hay, mixed grass haylage, ryegrass haylage and mixed forage chaff). Increasing the number of forages to five and six decreased the amount of eating time and increased the amount of standing behaviours observed, when compared to four forages. Introducing an adaptation period between diets would further improve this study however the latin square design would have prevented any consistent effects from previous diets affecting results gained. A further study is required to determine if the increased foraging behaviour observed would persist over a longer period and which forage combinations result in behaviours most similar to free living observations.

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# Did the horses that reached the final of the 2004 PAVO British Eventing Breeding Championships show a higher level of subsequent performance in British Eventing competition compared to those who did not qualify?

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**Introduction** The production of young sport horses for British Eventing (BE) competition requires huge investment in terms of time, money and effort. To ensure that such resources are not wasted, it is necessary to select the individuals that display the most talent and potential for an illustrious competitive career. In order to facilitate such selection, the BE Breeding Championships were introduced in 2003. Sponsored by feed manufacturers PAVO, the series is formed by a number of national qualifiers run within a selection of BE one-day events each year. Successful horses qualify for the championship final at the end of the eventing season. The objective of the series is to provide a showcase of the most talented young event horses in the UK for breeders, owners, riders and prospective buyers. The aim of the study was to establish whether 4, 5 and 6 year old horses that qualified for the 2004 PAVO BE Breeding Championship final performed better in BE competition during the following year of competition compared to horses of the same age that did not qualify for the final.

**Materials and Methods** In order to provide the greatest amount of data and standardised qualification methods, 2004 was selected as the Championship Year under investigation (labelled CY for analysis purposes). Horses' performances were monitored throughout 2005, the first year of competition after CY (identified as CY+1). Performance data of 4, 5 and 6 year old horses (n=14, n=29 and n=20 respectively) that completed the 2004 championship final were obtained from the BE website and formed the Elite study groups. Non-elite control groups were randomly selected from data of 4, 5 and 6 year old horses (n=14, n=31 and n=21 respectively) that completed a national qualifier during CY but did not progress to the championship final. The BE performance results of all Elite and Non-elite horses were analysed individually for each horse during CY+1. A system by which points were awarded for successful results in BE competition was developed in order to assess performance in a quantifiable manner, at all levels of BE competition. Points were allocated for placing in BE competition and normalised for the number of starters. The assessed variables of performance were average points per start in BE competition and the end year level of competition achieved. End year levels of competition were coded numerically to allow statistical analysis.

**Results** Horses within the Elite four year old group attained a mean of 1.5 points more per start than the Non-elite four year olds for CY + 1 (p=0.048) (Figure 1). The Elite five year old group scored a mean of 1.2 points more points per start than the Non-elite group (Figure 1). The difference between Elite and Non-elite horses was not, however, found to be significant (p=0.145). In the six year old age category, Elite horses attained a mean of 1.9 more points per competitive outing than Non-elite horses (p=0.013) (Figure 1). The end year levels of competition did not differ between Elite and Non-elite four year old horses, both groups competing at Pre-Novice level throughout CY + 1 (p=0.839) (Figure 1). The five year old Elite group displayed a mean end year level of Intermediate-Novice, two BE levels higher than the Non-elite horses who were found to be competing at a mean level of Pre-Novice (p=0.000) (Figure 1). Elite six year old competitors were shown to be competing at CCI** level on average, three BE levels higher than the Non-elite group who exhibited a mean end year level of Intermediate-Novice for CY+1(p=0.001) (Figure 1).

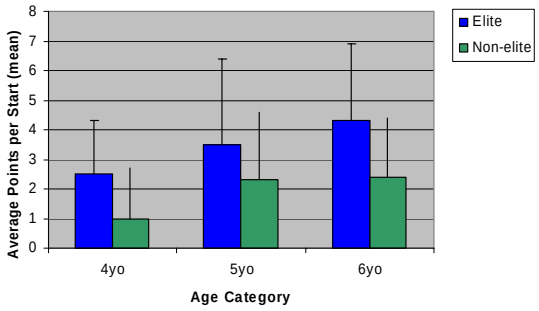


**Figure 1: Average Points per Start for 4, 5 & 6 year old horses during CY+1 (Y error bars indicate s.d)**

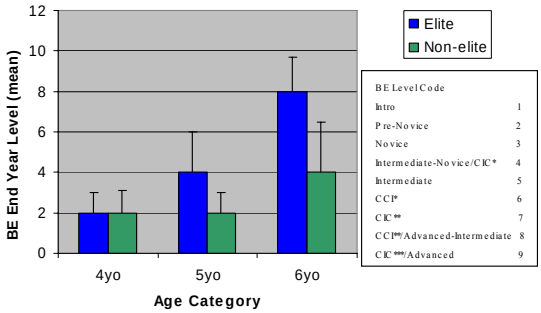


**Figure 2: End Year Level in BE competition of 4, 5 & 6 year old horses during CY+1 (Y error bars indicate s.d)**

**Conclusion:** A clear distinction in levels of performance was apparent between the Elite and Non-elite groups for all age categories. The Elite horses displayed a significantly higher number of average points per start than Non-elite horses. Elite competitors also exhibited a higher end year level of competition throughout all age categories. In conclusion qualification for the final of the PAVO championship indicates a horse will reach a higher level of competition compared to one that has not qualified. The influence of the rider was not assessed within the study. Further research of this type is required to establish whether a correlation exists between championship final placing and performance in BE competition. A longitudinal study of greater scope conducted over several years may help to develop and refine the series further in order to ensure that the most promising prospects for elite performance are selected and invested in for the future.

# The effects of different thawing methods on equine colostral IgG integrity and perseverance

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**Introduction** The equine placenta being epitheliochorial, is a barrier to immunoglobulins, which means that the foal is unable to receive passive immunity (Davies Morel, 2003) other than a small quantity of IgM which is synthesised in utero. McClure (as cited in McKinnon and Voss, 1993) suggests that the most significant component of passive immunity passed to foals, is that obtained from colostral immunoglobulins. When the foal ingests the colostrum and absorbs the antibodies therein, a temporary protection is provided until autogenous production of immunoglobulins reaches protective levels (McClure as cited in McKinnon and Voss, 1993). It is highly important that the foal receives colostrum within the first 24 hours and preferably within the first 12 hours of life (Davies Morel, 2003). This enables immunoglobulins to be absorbed by enterocyte cells lining the small intestine which are controlled by cortisol, and over time are replaced by ones which are unable to absorb proteins. For foals where colostrum is not readily available (for example, if the foal is orphaned at birth), then stored colostrum could be used. However, it is postulated that IgG integrity could be compromised by freezing, and consequently, thawing of the colostrum. The aim of this experiment is to evaluate five thawing methods for their ability to preserve IgG content of stored colostrum.

**Materials and methods** Colostral samples were obtained from 15 mares of varying breed, (5 thoroughbreds, 9 warmbloods and 3 fell ponies), and age (2 years to 20 years). Expected foaling date, actual foaling date, foaling time, sex of foal, BRIX percentage and g/l level were all recorded. A 30ml sample of colostrum was collected in a beaker from each mare immediately following parturition. It was ensured that the collection process in no way interfered with either the mare or the foal. The colostrum was then filtered so that debris was separated out. Each sample was then split into five 6ml replicates and using a colostrometer, the IgG level of each was recorded, as the % BRIX level (sugar refractometer correlated against IgG levels). Each replicate was then frozen at -20°C with T-type thermocouples. Twelve hours post-freezing, the colostrum was thawed by one of five methods adapted from Arguello et al. (2003). Method 1 - placed into a bowl of hot water at 45°C. Method 2 - Cold Storage. The sample was placed in a refrigerator set at 4°C. Method 3 - Room Temperature. The sample was placed on the work surface of a lab bench (28°C). Method 4 - Body Temperature. The sample was placed in a heat cabinet at 38°C. Method 5 – Microwaved - The sample was placed in the microwave for 15 seconds on high. For methods 1-4, thermocouples were attached to a computer and the temperature change was recorded using Picolog. When the sample appeared fully thawed (so that there were no ice crystals present) and the thermocouple reached the external environment temperature, IgG levels were tested again. Thermocouples were not used within the microwaved samples. Instead after the 10 seconds, the thermocouple was quickly placed inside the microwave to record the temperature trace.

**Results** Differences in %BRIX levels were analysed using ANOVA. It was found that there were significant differences, ($F$=0.002, P<0.05) between thawing method and %BRIX, with method five (microwaving), showing a reduced %BRIX (Figure 1). This method also resulted in the fastest thawing time. However, it was found that there were no differences between colostral quality and BRIX % for microwaved samples (P=0.121).

**Conclusions** It was established that BRIX % was not affected by the age of the mare, which is consistent with other published work (Kohn *et al*.,1989). The use of microwaving as a thawing method for equine colostrum appears to be the least affective method of preserving colostral IgG levels. Significant differences in the %BRIX content of microwaved samples were evident, although colostral quality was not affected to the same extent. This reduction in IgG levels, appears to be intrinsically linked to the length of time taken to thaw. The preceeding four thawing methods proved to maintain IgG levels adequate to prevent FPT (Failure of Passive Transfer). In the case of method 5, IgG levels dropped below 20%, and were therefore deemed unable to prevent FPT. Therefore, it can be concluded that with the exception of microwaving, all thawing methods examined were acceptable methods to employ in a practical situation. Should timing be critical, then method 3 – thawing at room temperature, appeared to elicit the quickest thawing time.

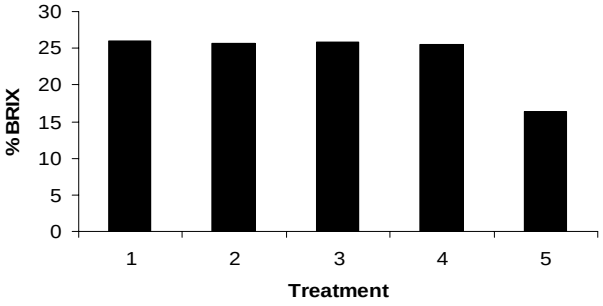


**Figure 1** Mean % BRIX for the 5 methods

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## Quantitative aspects of calcium absorption and excretion in growing horses

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**Introduction** Ca bioavailability has been of considerable interest. NRC (1989) expressed Ca requirement as total Ca but assumed an absorptive efficiency of 0.50. To improve the understanding of calcium absorption and excretion in horses, the present study analysed the quantitative calcium metabolism in animals fed different levels of calcium.

**Materials and methods** Three diets containing 2.4g of phosphorus/kg diet as DM basis and different levels of calcium (T1: 1.5; T2: 4.5 and T3: 7.5 g Ca/kg of diet as dicalcium phosphate) were offered to twelve growing horses (LW = 221±64 kg). True Ca absorption was determined by the isotope dilution technique. The animals were kept in metabolic cages for 21 days, designed for isotope studies, and each animal was given a single dose, comprising 30 MBq of $^{45}$Ca in 1mL of sterile isotonic saline, into the jugular vein. Blood samples were withdrawn from jugular vein at 24hs intervals for 7 days. From the time of injection, Ca balance was recorded daily for 7 days through total collection of feed, refusals, faeces and urine. The experiment was carried at the Nutrition Laboratory, according to recommendations of the Radiological Protection Service and the Animal Experimentation Ethic Committee at CENA. All parameters ($CaFEC_{TOT}$: total faecal Ca excretion; CaURI: Ca excreted in urine; $CaFEC_{ENDO}$: endogenous faecal Ca; CaABS: absorbed Ca; CaRET: retained Ca; and CaING: ingested Ca) were normalized according to dry matter intake (DMI) as suggested by Bravo et al. (2003). Linear and quadratic effects of ingested Ca on the other parameters were evaluated by regression procedure of SAS system (SAS, 2000).

**Results** No quadratic effect was observed. Regression equations are presented in Table 1. Total faecal calcium excretion ($CaFEC_{TOT}$) was affected by calcium ingestion (CaING), representing 0.33 of ingested calcium excreted in faeces on average. This is in accordance to van Doorn et al. (2004). Endogenous faecal Ca excretion was practically constant, representing less than 1g Ca/kg DMI and the same was observed for urine calcium excretion (less than 0.06 g Ca/kg DMI). Schryver et al. (1970) reported for horses that the endogenous loss was not related to intake (mean 2.2 g/day). CaABS was 0.78 of Ca intake and 0.61 of Ca intake was retained. Excessive intakes of Ca reduce the true availability (Hintz, 1990) and for ponies fed a level near the requirement the absorption was 0.70. In the present study the highest Ca level fed the animals was not in excess, since the true absorption and the retention were increased as Ca level increased.

**Table 1** Linear regressions between CaING (*x*) and other parameters (*y*), normalized by dry matter intake (DMI)

| *y* | slope | | | intercept | | | $R^{2\,(c)}$ | RMSE [(d)] |
|---|---|---|---|---|---|---|---|---|
| | value | s.e.m. [(a)] | *P* [(b)] | value | s.e.m. [(a)] | *P* [(b)] | | |
| $CaFEC_{TOT}$/DMI | 0.327 | 0.0511 | <0.0001 | 0.153 | 0.2903 | 0.61 | 0.803 | 0.463 |
| $CaFEC_{ENDO}$/DMI | 0.054 | 0.0389 | 0.20 | 0.513 | 0.2209 | 0.04 | 0.160 | 0.352 |
| CaURI/DMI | 0.067 | 0.0276 | 0.04 | -0.137 | 0.1568 | 0.40 | 0.370 | 0.250 |
| CaABS/DMI | 0.777 | 0.0710 | <0.0001 | 0.253 | 0.403 | 0.54 | 0.923 | 0.643 |
| CaRET/DMI | 0.606 | 0.0555 | <0.0001 | -0.016 | 0.3152 | 0.96 | 0.923 | 0.502 |

[(a)] s.e.m.: standard error of means
[(b)] *P*: probability
[(c)] $R^2$: determination coefficient of the regression
[(d)] RMSE: root MSE

**Conclusions** Although the level of 7.5gCa/kg DM in diet is considered high for the animals in the present experiment the highest Ca absorption and retention was obtained. It can be inferred that excess of Ca in the diet is reflected directly in Ca voided in faeces, which might contribute to environmental pollution. Isotope dilution technique permits us to determine more precisely Ca requirement of the animal, and thus obtain more adequate values of dietary recommendations without an excessive safety margin.

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# An investigation into daily fluctuations in faecal pH in healthy equine

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**Introduction** Laminitis is often recognised as one of the most common causes of lameness in horses. This painful condition has obvious far-reaching implications for the welfare of affected animals, yet, despite the prevalence and seriousness of laminitis, the exact aetiology of the disease has still to be unanimously identified. It is generally accepted that acidosis within the hind gut of the horse is a predisposing factor to the onset of laminitis (Bailey *et al*., 2003). Fast digestive transit times and low production rates of α-amylase, result in the horse being relatively inefficient at the breakdown of starch within the small intestine. Rapid fermentation of cereal starch in the caecum and dorsal colon will ultimately yield lactic acid and can culminate in changes to the caecal bacterial populations (Bailey *et al*, 2003). Rising lactic acid levels, coupled with a drop in pH will result in the development of acidosis. It is believed that a window of opportunity exists between the occurrence of acidosis and the development of the symptoms associated with laminitis (Al Jassim, 2005) in which changes to diet could be made, thus preventing the eventual onset of the disease. It has been established that faecal pH is intrinsically linked to caecal pH, and studies reporting diet-induced pH changes in both horses (McLean *et al*., 2000) and pigs (Cahn *et al*., 1998), have shown close correlation between faecal and caecal pH. It is therefore postulated that faecal pH may be an indication of acidotic conditions within the hindgut of the horse and a faecal test to indicate acidotic status would obviously be of benefit. The aim of this study was to investigate optimal collection times of faecal samples destined for pH analysis, and to establish whether time of feeding significantly altered faecal pH, thus enabling further work to be carried out on the development of a faecal test for acidosis.

**Material and method** Nine riding school horses of varying breeds were selected at random for use in the experiment (3 Warmblood; 2 Thoroughbred × Warmblood; 2 Welsh; 2 Fell). All horses were fed on *ad libitum* hay only diet, which was provided at 8am. The experiment consisted of three sampling periods, each lasting a total of 16 hours (7am – 11pm). During the sampling periods, faecal samples were collected from all horses ever hour to enable fluctuations in pH to be ascertained. If horses defecated more than once within the 1 hour period, then samples where combined and sub-sampled so that a combined pH value for the hour was achieved. Upon collection, the samples were immediately tested for pH. 20g of faeces were mixed in 200ml of water for two minutes. The pH was then measured using a pH meter (Jenway 3051, Fisher Scientific, UK), calibrated against pH 7 and pH 4 buffers. The values were later analysed in SPSS using a Wilcoxon's test.

**Results** The results showed that mean pH for all animals increased, albeit insignificantly throughout the day, the range for each horse varying from 0.48 to 1.09 difference in pH. As pH is measured on a logarithmic scale, this difference can prove to be significant from a practical standpoint. Very highly significant differences ($P<0.001$) were found between pH values measured prior to, and after, the morning feed (Table 1).

**Conclusions** All horses maintained a faecal pH above pH 6. It is accepted that horses exhibiting a caecal pH of below 6, can be regarded as suffering from sub-clinical acidosis (Radicke *et al*., 1991). As faecal pH is regarded as an indicator of caecal pH, it can be assumed that all horses maintained healthy gut function throughout the period of the trial. All horses, except one, showed a very highly significant ($P>0.001$) increase in mean faecal pH after the consumption of food. As hay was fed *ad libitum*, it is possible to assume that horses ate throughout the day, which would account for the generalised increase in pH values collected throughout the day. It is therefore possible to conclude that faecal pH measured later in the day, presumably after the consumption of food, will exhibit higher values, and may be considered as falsely positive, than those taken before daily meal provision. Therefore, in order to obtain accurate fasting, faecal pH values, it is necessary that samples be collected prior to the morning feed.

**Table 1** Mean pH differences before and after the morning feed for all horses ($P>0.001$)

| Horse | Mean faecal pH prior to feed | Mean faecal pH after feed | s.e.d. | sig |
|---|---|---|---|---|
| 1 | 6.91 | 7.27 | 0.180 | *** |
| 2 | 6.62 | 7.08 | 0.230 | *** |
| 3 | 7.13 | 7.03 | 0.050 | ns |
| 4 | 6.67 | 7.53 | 0.430 | *** |
| 5 | 7.01 | 7.07 | 0.030 | *** |
| 6 | 7.09 | 7.29 | 0.035 | *** |
| 7 | 6.88 | 7.09 | 0.105 | *** |
| 8 | 6.75 | 7.22 | 0.235 | *** |
| 9 | 6.70 | 7.01 | 0.155 | *** |

* Very highly significant difference ($P>0.001$)

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# The effect of hoof lesion severity on stride characteristics of early lactation Holstein dairy cows measured using locomotion scoring and computerised motion analysis

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**Introduction** Kinematic assessment of gait has been widely used in sports and equine science, this method gives objective and accurate information on the movement of the subject. Most locomotion scoring systems incorporate stride length however this is subjective and is difficult to compare observers. Kinematic studies have shown that cows with sole ulcers have a shorter stride length than those showing no hoof lesions (Flower *et al*, 2005). Telezhenko and Bergsten (2005) found a similar effect with moderately lame cows having a shorter stride length measured objectively from footprints than non lame cows. Increased locomotion score can also affect the tracking distance of the animal (Telezhenko and Bergsten, 2005). The aim of this study was to assess the effect of increasing locomotion score on stride length and tracking distance of Holstein dairy cows in early lactation.

**Materials and methods** Twenty five lactating Holstein dairy cows (6 primiparous and 18 multiparous (parity 2- 6) mean ± SE, parity 2.6 ± 0.28) were recruited to the study within 7 days post partum (6 ± 0.7 days). A digital video camera (Cannon PAL MV690) was placed 15m perpendicular to an alley. The alley was 1.6m wide and the field of view captured using the video camera was 4.5m. Filming took place following morning milking. A yellow cardboard marker 3cm diameter was glued onto the fore and hind fetlock on the condoyle on the distal end of the carpal bone at the same height as the sesamoid bone. Area surrounding the marker was highlighted by animal marker (Richey Sprayline). The images were then digitised using Simi Motion Analysis Software (SIMI Reality Motion Systems GmbH, Postfach 1518, D-85705 Unterschleissheim). Hind and fore stride length were calculated separately. Stride length was determined as the distance between the cannon appearing straight and the next occurrence of the cannon being straight for both the fore and hind limbs. Tracking distance was calculated as the distance between the fore foot being placed on the ground and the ipsilateral hind foot being placed on the ground. A cow with perfect foot placement would have a tracking value of 0cm, a negative result indicates that the cow fails to place the hind foot in the place of the fore foot and therefore is short-striding. At each filming session at least three strides were analysed. All cows were filmed from the left and all measurements taken on the left fore and hind feet. All cows were locomotion scored and severity of lesions assessed 8 weeks after filming using the method of Flower and Weary *(*2006). Stride data and tracking was tested using the Analysis of Variance and Kruskal-Wallis tests respectively using Genstat (Version 8, Lawes Agricultural Trust).

**Results** Hind stride was significantly longer ($p<0.05$) for cows visually defined as having a normal gait (locomotion score 1) compared to those that showed moderate lameness (score 3). No significant difference was found between cows with score 1 and 2 or 2 and 3. There was no significant effect of locomotion score on fore stride length. There was a tendency for cows with moderate lameness to have a more negative tracking distance ($p=0.12$). There was a significant relationship between locomotion score and lesion severity with locomotion score 3 cows having significantly higher ($p<0.01$) lesion severity score than those with locomotion score 1 and 2. Lesion severity was not different between cows with locomotion score 1 and 2. Lesion distribution was different between hind and fore feet; 80% of lesions occurred in the hind feet, of all lesions on the hind feet 60% were found on the left hind foot.

**Table 1** Stride characteristics of early lactation dairy cows with different locomotion scores

| | Locomotion Score 1 | Locomotion Score 2 | Locomotion Score 3 | s.e.d. | Significance |
|---|---|---|---|---|---|
| Fore stride (cm) | 180 | 175 | 162 | 0.1 | NS |
| Hind stride (cm) | 174 [a] | 165[ab] | 150 [b] | 0.1 | * |
| Tracking (cm) | 2.1 | 0.4 | -10.6 | 0.05 | NS |
| Lesion Severity | 0.67[a] | 0.89[a] | 2.40[c] | 0.426 | ** |

[abc] different superscripts indicate significant differences between means within rows. a vs b $p<0.05$, a vs c $P<0.01$

**Conclusions** These results suggest that more severe lesions lead to a shortening of stride in the hind limb. The majority of lesions occurred on the hind feet which may explain why there was no difference in fore stride length. Tracking distance is also affected by lesion severity with the most severe lesions more likely to have a negative tracking value than those with less severe lesions. This method of measuring stride characteristics shows potential for the detection of severe hoof lesions.

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# Using different levels of hydrolyzed bagasse pith in early lactation Holstein cows nutrition

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**Introduction** In recent years sugarcane had been farmed in large quantities in Iran especially in Khoozestan state due to proper climate. By-products that remain after processing of sugarcane are Lignocellulosis materials contain more than 20% lignin. 700000 ton bagasse pith is produced yearly at Khoozestan that might be used in animal nutrition because industrial usage is limited. Supplying fiber requirement of ruminants in Iran because of hot and dry climate needs attention to new sources of fiber. Bagasse had been used intact in some countries or just with molasses and urea in fattening farms. Bagasse had been used in low milk production cows (1). A new technology (Steam treatment) had open new zones to apply bagasse as steam treated bagasse in ruminant nutrition and had been shown that it is more digestible (2). This study was conducted to use hydrolyzed bagasse pith in high producing dairy cows in early lactation.

**Materials and methods** In order to investigating effects of wheat bran substitution with varying amounts of hydrolyzed bagasse pith (No bagasse pith or 0, 4, 8 and no wheat bran or 12% DM-base) an experiment conducted using 8 early lactation Holstein cows (32±2.5) in 4 periods of 21 days (include: 14 days adaptation and 7 days sampling). TMR was fed to cows two times daily (Based on NRC 2001 nutrient requirement). Milk samples obtained analyzing its composition. DMI and milk produced was recorded daily. Rumen samples obtained via stomach tube (Oral), 2 hours after morning feeding and pH determined immediately using pH-meter. Statistical design was Latin square with repeated squares. Data analyzed using SAS 6.12. Means compared using Duncan test ($P<0.05$).

**Results** As can be seen in table 1 using hydrolyzed bagasse pith had significantly influenced milk yield and DMI between treatments ($P<0.05$). DMI decreased with increasing amounts of hydrolyzed bagasse pith. Maximum milk production takes place at no bagasse pith and minimum at 8 and 12 % bagasse pith. Fat % was significantly altered with treatments and was highest at 12% bagasse pith ($P<0.05$). Correction of milk with fat (FCM 4%) didn't change milk amounts except at 12% that increase milk yield from 36.34 to 37.97. Anyway it was significant ($P<0.05$). Protein %, Lactose %, SNF %, TS %, SCC and rumen pH had no differ between treatments. Fat and protein content of milk was influenced by treatments ($P<0.05$).

**Table 1** Effects of different levels of hydrolyzed bagasse pith on performance of early lactating Holstein cows

| Items | Treatments (% of Bagasse pith) | | | | SEM |
|---|---|---|---|---|---|
| | 0% pith | 4% pith | 8% pith | 12% Pith | |
| DMI (Kg) | 26.53 [a] | 25.18 [ab] | 22.68 [ab] | 21.65 [b] | 0.987 |
| Daily milk (Kg) | 39.30[a] | 37.57 [ab] | 36.75 [b] | 36.34 [b] | 0.734 |
| FCM 4% (Kg) | 39.92 [a] | 37.59 [ab] | 36.94 [b] | 37.97 [ab] | 0.927 |
| Fat (%) | 4.13 [ab] | 3.98 [b] | 4.04 [b] | 4.28 [a] | 0.092 |
| Protein (%) | 2.91 | 2.76 | 2.88 | 2.89 | 0.032 |
| Fat (Kg) | 1.61 [a] | 1.50 [ab] | 1.48 [b] | 1.56 [ab] | 0.047 |
| Protein (Kg) | 1.14 [a] | 1.03 [b] | 1.06 [ab] | 1.05 [ab] | 0.027 |
| Lactose (%) | 4.61 | 4.48 | 4.66 | 4.62 | 0.047 |
| SNF (%) | 8.21 | 7.91 | 8.20 | 8.22 | 0.084 |
| TS (%) | 12.35 | 11.75 | 11.36 | 12.50 | 0.318 |
| SCC (x1000) | 332 | 293 | 317 | 298 | 20.053 |
| Rumen pH | 6.64 | 6.41 | 6.38 | 6.43 | 0.087 |

**Conclusions** It seems that in conditions that milk price is determined by milk volume addition of hydrolyzed bagasse pith will reduce the milk production and just up to 4 % hydrolyzed bagasse pith could substitute in diet instead of wheat bran. But if the milk price is determined by milk composition then we could substitute wheat bran by hydrolyzed bagasse pith up to 12 %. Rumen pH shows stable rumen environment in all treatments.

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# Plasma essential amino acids concentrations of early lactating Holstein dairy cows fed diet containing raw or roasted Iranian soybean

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**Introduction** Whole soybean has 40-42 percent CP and used as high energy-protein supplement for early lactation dairy cows. However, the protein is highly degradable, so small amounts of amino acids can be reached to small intestine to meet high amino acid requirements of early lactating cows. Therefore, various chemical and physical treatments have been suggested to decrease ruminal protein degradability of soybeans. The practical use and application of any one method to lower ruminal feed degradability is dependent not only on its efficacy but also on its cost effectiveness, safety and ease of application. For these reasons, heat treatment is the most commonly used physical method (Plegge *et al*., 1985). The purpose of this study was to determine how roasting of soybeans affect plasma essential amino acid concentrations in early lactation cows.

**Materials and methods** Iranian variety *(Sahar)* of soybean was used in the present study. The soybeans were fed into a turning cylindrical tunnel with a flame and bower at its end, so that they were exposed to burning air. The temperature of the soybeans exiting the roaster was 130 to 135 ºC. After that, they immediately placed in barrels, without cooling, and covered with canvas for about 45 minutes (steeping), then cooled. Fourteen Holstein dairy cows were used in this study (based on complete randomized design with 3 treatments). At the beginning of the trial the cows had a mean lactating stage of 16.9 days (s.d. 6) and mean milk yield 33.2 Kg/d (s.d. 3.39). Three total mixed rations based on concentrate and forage (0.39:0.62 dry matter (DM) basis) were offered individually throughout the study to groups 1 to 3 and had the following composition: NE1 1.61 M cal/Kg DM, CP 176 g/Kg DM, RUP 65.2 g/Kg DM, RDP 110.3 g/Kg DM, NDF 305 g/Kg DM, Ca 6 g/Kg DM, P 4.5g/Kg DM. Rations 1 to 3 were included by roasted soybean (RoS), raw soybean (RS) or soybean meal (SM), respectively. During last week of experiment ($6^{th}$ week) blood samples were taken from vein tail vessel, 4h after feeding), then, plasma essential amino acids concentrations were analyzed using AccQ (Tag method with 717 HPLC). Data were analysed using general linear model procedure of SAS using complete randomized design (SAS, 2000). Mean of treatments were compared by Duncan method and reported as significant while $P< 0.05$.

**Results** Plasma essential amino acid concentrations are shown in Table 1. There was Significant differences between diets ($P< 0.05$) when individual blood essential amino acid concentration was considered.

**Table 1** Plasma essential amino acid concentrations (mmol/L) of early lactating Holstein dairy cows fed diets containing roasted soybean, raw soybean and soybean meal

| | Treatments* | | | |
|---|---|---|---|---|
| Essential amino acid | ROS | RS | SM | SEM |
| Arginine | $16.7^{a}$ | $14.5^{b}$ | $14.3^{b}$ | 0.98 |
| Histidine | $5.5^{a}$ | $4.4^{b}$ | $4.6^{b}$ | 0.35 |
| Isoleucine | $14.6^{a}$ | $13.3^{b}$ | $13.1^{b}$ | 0.66 |
| Leucine | $12.5^{a}$ | $14.1^{b}$ | $14.0^{b}$ | 0.71 |
| Lysine | $6.8^{a}$ | $5.7^{b}$ | $6.0^{b}$ | 0.37 |
| Methionine | $3.4^{a}$ | $2.8^{b}$ | $3.0^{b}$ | 0.06 |
| Phenylalanine | $4.3^{a}$ | $4.6^{b}$ | $5.3^{b}$ | 0.38 |
| Threonine | 12 | 11.7 | 11.3 | 0.74 |
| Valine | 19.3 | 18.8 | 20.2 | 1.73 |

* RoS: Roasted soybean, RS: Raw soybean, SM: Soybean meal

**Conclusions** Roasting caused to decrease the soluble nitrogen and protein degradability (Fathi *et al.* 2006). The results of the present study showed that the roasting of soybean is an effective processing method for increasing of blood essential amino acid concentrations of early lactating Holstein cows. When individual essential amino acids were consider, the concentration of all amino acids, except methionine and phenyalanine, were significantly affected by the roasting ($P< 0.05$).

**Acknowledgments** Financial support of Excellence Centre for Animal Science is gratefully acknowledged.

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Plegge, S. D., Berger, L. L. and Fahey, G. C. 1985. Effect of roasting temperature on the proportion of soybean meal nitrogen escaping degradation in the rumen. *Journal of Animal Science*. **61:**1211-1218.

# Effect of diets containing rapeseed meal or soybean meal on lactating Holstein cow responses

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**Introduction** Rapeseed meal (RSM) is becoming an increasingly important source of protein for ruminants and nonruminants in the world. In some studies, inclusion of RSM instead of other protein sources such as SBM in dairy cow diets caused to increase milk production (Emanuelson, 1994). There was a positive association between the milk protein content and content of RSM in the ration (Emanuelson *et al*., 1993). Present experiment was carried out to evaluate the effect of inclusion of RSM or SBM in a basal diet on the responses of lactating Holstien dairy cows.

**Materials and methods** Fourteen multiparous and primiparous Holstein cows averaging 49 ± 13.21 days in milk were assigned in a completely randomised design for eight weeks. The animals were housed in a tie-stall barn and given *ad libitum* a total mixed ration. The basal diet was consist of 12.36% lucerne hay, 24.39% maize silage, 11.39% barley, 13.51% maize, 10.58% cottonseed and 7.02% sugar beet pulp. Treatment 1 (T1; 8.38% wheat bran, 10.95% SBM) and Treatment2 (T2; 6.91% wheat bran, 10.85% RSM plus 1.41% SBM) was calculated to achieved a same amount of CP and ME. Experimental diets were formulated based on AFRC (1995) recommendations. Dry matter intake (DMI), milk production and composition were determined weekly. Faecal pH was recorded in weeks 4 and 8. Body condition score (BCS) was also determined in weeks 4 and 8. The data were analyzed using the MIXED procedure of SAS (2001) for a completely randomized design with repeated measures. The model contained the effects of treatment, time, cow within treatment and the interaction of treatment by time.

**Results** The diet had no significant effect on milk fat and lactose, and milk urea nitrogen, faecal pH and BCS (Table1). DMI was significantly lower for T2 compared with T1 ($P< 0.05$). Milk protein and solid non fat (SNF) were significantly higher in cows fed T2 compared with those fed T1 ($P< 0.05$).

**Table 1** Effect of diet containing RSM or SBM on performance of lactating dairy cows

| Items | T1 | T2 | SEM | *P-value* |
|---|---|---|---|---|
| DMI (kg/d) | 20.5 | 19.9 | 0.21 | 0.03 |
| Milk yield (kg/d) | 38.0 | 37.9 | 0.41 | 0.91 |
| Milk fat (g/kg) | 25.8 | 28.1 | 0.12 | 0.20 |
| Milk protein (g/kg) | 27.6 | 28.9 | 0.03 | 0.01 |
| Milk lactose (g/kg) | 45.3 | 47.4 | 0.08 | 0.10 |
| Milk SNF (g/kg) | 81.8 | 86.2 | 0.12 | 0.03 |
| Milk urea nitrogen (mg/dl) | 16.5 | 16.2 | 0.37 | 0.56 |
| Faecal pH | 6.9 | 6.9 | 0.03 | 0.40 |
| BCS | 2.3 | 2.3 | 0.12 | 0.80 |

T1: diet containing 10.95% SBM, T2: diet containing 10.85% RSM+1.41% SBM

**Conclusions** The decline of the DMI when RSM was included in the diet might be attributed to the protein degradability of this protein source. In the present study, the increase in milk protein of cows fed T2 compared with the animals fed T1 might be due to the RSM protein nature which was shown to be more rapidly degradable by rumen microorganisms (Khorasani *et al.,* 1989). Milk composition and production responses achieved in the present experiment indicated that the inclusion of RSM instead of SBM did not adversely affect on animal performance and can be used in dairy diets in order to decrease feed cost.

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# Investigating the relationship between protein-fat difference and milk yield and somatic cell counts of Iraninan Holstein dairy cows

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**Introduction** Milk quality control is one of the most important management tools used on dairy farms. Periodic recording and accurate analysis of data is needed. Management decisions and genetic evaluations are based on these data (Miglior, 2002; Wiggans, 1985). Good nutrition management is important for economical production (improvedmilk yield and protein% and fat%) and health of animals (Schroeder, 1996). It is possible to evaluate the management system and nutrition program in a herd according to the protein% and fat% of milk. Cows that produce low milk with high fat are generally weak or have a low DMI. Low milk fat is caused by metabolic disorders or unbalanced feed composition. Low milk protein is due to lack of energy in diet (JafariKhorshidi, K. and J. Soltaniha. 1382). The aim of this study was to investigate the effects of protein % to fat % difference on milk production of Holstein dairy cows.

**Materials and Methods** Milk (kg), fat%, protein% and somatic cell count (SCCx1000) records from north, south and Razavi Khorasan states of Iran (59044 record) were adjusted using days in milk, parity, milking times, year and month of recording. Data obtained from Jihad-e Agriculture Organization of Khorasan state of Iran, Animal Science section. Protein-fat difference was determined using fat% and protein% records based on grouping (cows were divided to 8 groups: protein fat differences; less than -1, -0.99 to -0.5, -0.49 to 0, 0.01 to 0.25, 0.26 to 0.50, 0.51 to 0.75, 0.76 to 1.00 and more than 1.01). Data were analyzed using SAS v.6.12 based on a completely randomized design. Means were compared using the Duncan multiple range test ($P<0.01$).

**Results** Table 1 shows that protein fat difference can influence milk yield ($P<0.01$). Also it has an effect on SCC in milk ($P<0.01$). When milk fat is more than protein (negative groups), it shows that milk yield is lowest and gradually by decreasing fat content and constant amount of protein, milk yield increased (Negative relationship between milk yield and fat percentage). When fat content is more than 0.5 percent below protein, again milk begins to reduce. At a protein to fat difference of about 0.75, rate of milk reduction increases. This difference between milk protein and fat is an indicator of acidosis (Naserian, et al 2002) although rumen pHwas not measured in this study. 31.03 percent of records indicate cows that may be affected with some level of acidosis. This adversely affects milk production. The trend in SCC followed that of milk production, unless most SCC takes place at protein fat difference about 0.51 to 0.75.

**Table 1** Effect of protein fat difference on milk yield and somatic cell count of Holstein dairy cattle

| Items | Grouping (Protein %-Fat %) | | | | | | | | SEM |
|---|---|---|---|---|---|---|---|---|---|
| | < -1.00 | -0.99 to -0.5 | -0.49 to 0 | 0.01 to 0.25 | 0.26 to 0.50 | 0.51 to 0.75 | 0.76 to 1.00 | >1.00 | |
| Milk (Kg) | $25.63^{f}$ | $25.97^{e}$ | $26.86^{c}$ | $27.94^{b}$ | $28.95^{a}$ | $28.90^{a}$ | $28.15^{b}$ | $26.58^{d}$ | 0.032 |
| SCC[1] (x1000) | $297^{f}$ | $314^{e}$ | $317^{de}$ | $325^{c}$ | $322^{dc}$ | $342^{a}$ | $335^{b}$ | $279^{g}$ | 0.839 |

Means with different characters are significantly different ($P<0.01$)

[1]Somatic Cell Counts

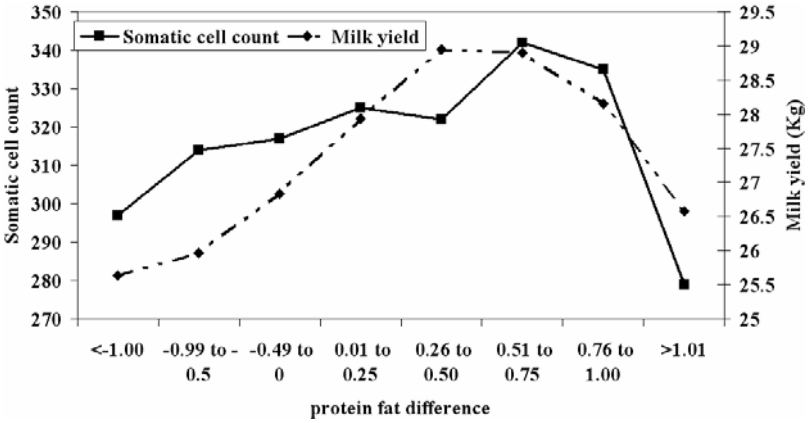


**Figure 1** Effect of protein fat difference on milk yield and somatic cell count of Holstein dairy cattle

Conclusions According to these data it seems that efficient use of monthly records which usually exist in herds are a suitable tool to investigate herd nutrition and may be a good indicator of distribution of acidosis in herds because of the high correlation between differences in milk fat and protein percentages and incidence of acidosis.

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# Investigation into the substitution of alfalfa with sugar beet pulp on milk yield and composition of Holstein cows

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**Introduction** The non-NDF carbohydrates (NFC) are important sources of energy in the ration of high producing cows. The fibre must be proper quality and particle size to insure maximum DMI, optimal chewing activity, normal ruminal fermentation, and milk fat percentage. The NRC (1989) recommends 25 to 28% NDF in the rations of lactating cows; a minimum of 75% of the NDF should come from forages. These recommendations provide no adjustment for the physical effectiveness of the fibre, interactions among fibre sources and non fibre carbohydrates, or animal characteristics that may influence ration design. Few data are available to document the effect of the substitution of by- product NDF for forage NDF; our objective was to determine the effect of the substitution of alfalfa NDF from sugar beet on DMI, milk yield and composition, chewing activity, faecal and rumen pH, and apparent digestibility of DM.

**Material and methods** Twelve multiparous Holstein cows in mid lactation randomly allocated to four treatments in completely block design with 30days periods. Cows were blocked on the basis of parity, 3 blocks (cow), 4 treatments; Experiment was carried in strawed pen measuring 24 * 2m. Cows were fed total mixed diets twice daily for add libitum intake and were milked twice daily prior to feeding. Diets were: first diet (0, 50), second diet (7, 43), third diet (14, 36) and forth diet (21, 29) percent of sugar beet pulp and alfalfa in whole diet, respectively (table 1). Diet was isonitrogen (17.1% CP) and isoNDF (35%). Cows were fed for 30d individually and the last week of experiment used for sample collection.

**Table1** Chemical composition of the experimental diets

| | Treatments (DM %) | | | |
|---|---|---|---|---|
| Chemical analyses (%) | 1 | 2 | 3 | 4 |
| DM | 89.87 | 84.22 | 78.60 | 72.95 |
| Cp | 17.16 | 17.06 | 17.13 | 17.13 |
| NEl(mj/kg of DM) | 1.580 | 1.600 | 1.610 | 1.640 |
| NDF | 35.10 | 34.90 | 34.90 | 34.70 |
| NDF From forage | 23.00 | 19.80 | 16.60 | 13.40 |
| PeNDF* | 25.10 | 23.20 | 21.30 | 19.30 |
| NFC** | 40.60 | 41.60 | 42.10 | 42.90 |

*physically effective NDF **non fibre carbohydrate

Feed intake was measured daily, and diets were sampled weekly. Milk production was recorded daily and immediately were analyzed (Milk Scan 133B) for determining their composition. Apparent digestibility of dry matter was performed according to the method of AIA. Faecal sample were collected from the colon 3 times and frozen (-20ÂºC) until analyses. Rumen content was collected, 3 times, 3h after daily feeding by using oesophagus tube. The pH of each faecal and rumen samples were measured and recorded. Chewing activity was monitored for one continues 24-h period by observation of each cow once every 5 min.

**Results and conclusion** There were no significant differences between DMI ($p<0.01$), milk yield, milk yield adjusted to 4% fat, protein yield and milk fat. However milk and milk protein yield were higher for cows fed the low forage diets (SBP=sugar beet pulp) than for cows fed high fibre alfalfa diet. The fibrous components of SBP highly degradable in the rumen, because they are low in lignin and high in pectin, which may account for the relatively high digestible energy value of SBP (table 1).DMI was grater for cows fed SBP than for cows fed alfalfa, however this differences was no significant ($p<0.01$).Reduction of particle size of SBP than forage resulted in an increasing rate of passage of particulate matter from the rumen, a decrease in the digestibility of OM, and an increase in voluntary intake.

**Table 2** Milk yield and composition and DMI and rumination

| | DIETS | | | | |
|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **SE** |
| Milk yield (kg) | 15.080 | 16.140 | 16.49 | 17.070 | 1.150 |
| Milk protein (%) | 3.1900 | 3.2900 | 3.550 | 3.6200 | 0.245 |
| 4% FCM kg/d | 13.220 | 14.190 | 14.63 | 15.210 | 0.960 |
| Milk fat (%) | 3.1800 | 3.2000 | 3.250 | 3.2800 | 0.430 |
| DMI kg/d | 16.080 | 16.630 | 17.02 | 17.280 | 1.010 |
| Chewing (min/d) | $853.33^{a}$ | $793.33^{b}$ | $765.0^{bc}$ | $743.33^{c}$ | 13.42 |
| Rumination (min/kg DMI) | $29.010^{a}$ | $27.270^{ab}$ | $23.72^{bc}$ | $22.570^{c}$ | 1.870 |
| Rumen pH | 6.03 | 6.20 | 6.23 | 6.25 | 0.489 |

a, b, c, d, figures with different superscript differ significantly $p<0.01$

The time cows spent eating and ruminating (total chewing time) is a measure of the physically effective fibre value of feed. Eating time was lower for SBP than for alfalfa (table 2). The longer total chewing time might be attributable to the greater percentage of alfalfa haylage in diet 1. Rumination /kg of DMI were highest for diet 1 and lowest for SBP. The partial size of SBP caused reduced rumination time /kg of DMI, but increase the intake of DM. SBP supported desirable rumen pH of the cows which fed the SBP.

# Physically effectiveness of beet pulp in dairy cows 2: Intakes, digestibility and chewing activity, and performance of Holstein dairy cows

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**Introduction** Many non-forage fibrous sources (NFFS) such as beet pulp (BP) contain more NDF than some forage sources, and have been successfully used in dairy rations to replace a portion of the effective or physical effective fiber (peNDF) that is normally provided by forages. However, BP has limited forage replacement value and effectiveness factor of 0.40 (fraction of NDF) versus 1.0 for forages (Mertens, 1997). In addition, Mertens (1997) suggested that the physically effectiveness factor of ground and pellet were 0.40 and 0.30, respectively. However, the effects of BP when substituted with different feed sources in TMR are variable that depend on chemical composition, types and physical characteristics. The objective of this experiment was to measure the effects of three types of BP on intakes, digestibility, and chewing activity and performance of Holstein dairy cows.

**Materials and Methods** Nine multiparous Holstein cows (DIM =56 ± 5d) were assigned randomly to three treatments of a 3×3 Latin square change over design in three 21d periods (14d for adaptation, 5d for DMI and feces collection, 2d for chewing activity measurement, and milk sampling; Teimouri Yansari et al., 2004) to evaluate the effects of the three types of beet pulps on intakes, digestibility, ruminal kinetics, chewing activity and performance. Treatments were diets containing 12 % of DM fine dried (T1); normal dried (T2) and pelleted (T3) sugar beet pulp in TMR. The diets had similar chemical composition and contained 20, 20, 35, 7, 12, 5.5, 0.3, 0.1, and 0.1% of DM alfalfa, corn silage, barley, soyabean meal, sugar beet pulp, wheat bran, dicalcium phosphate, vitamin premix, and salt, respectively. Experimental data were analysed using the PROC MIXED of SAS® (1998) as a 3×3 Latin square change over design and differences between treatments were separated by Duncan at alpha =0.05). The data of ruminal pH and NH3 concentration over different sampling time were analysed while including hour of sample as a repeated measurement.

**Results and Discussion** The summary of results are represented as Table 1.

**Table 1** Summary results of experiment[1].

| Item | Total mixed ration contained beet pulp | | | SEM | P-Value |
|---|---|---|---|---|---|
| | T1 | T2 | T3 | | |
| DMI (kg/d) | 24.16 | 24.35 | 24.64 | 0.717 | NS |
| $peNDF_{>1.18}$(kg/d) | $5.26^{c}$ | $5.69^{b}$ | $6.34^{a}$ | 0.069 | *** |
| $PeNDF_{>8}$(kg/d) | 3.51 | 3.52 | 3.57 | 0.124 | NS |
| $peNDF_{PSPSnew}$(kg/d) | $5.10^{c}$ | $5.96^{b}$ | $6.18^{a}$ | 0.021 | ** |
| pH | $6.32^{c}$ | $6.44^{b}$ | $6.59^{a}$ | 0.012 | ** |
| $N\text{-}NH_3$[2] (mg/dL) | $14.08^{b}$ | $14.45^{ab}$ | $15.03^{a}$ | 0.111 | * |
| Total VFA concentration (m*M*) | $128.82^{a}$ | $120.32^{b}$ | $115.4^{c}$ | 1.26 | ** |
| Acetate (mol/100 mol) | $74.03^{a}$ | $72.63^{b}$ | $74.71^{a}$ | 0.39 | ** |
| Propionate (mol/100 mol) | $15.01^{b}$ | $16.13^{a}$ | $14.04^{c}$ | 0.46 | ** |
| Butyrate (mol/100 mol) | 7.90 | 8.03 | 8.00 | 0.57 | NS |
| Acetate: propionate | $4.34^{b}$ | $4.51^{b}$ | $5.32^{a}$ | 0.14 | ** |
| Lactic acid (m*M*) | $0.34^{a}$ | $0.315^{b}$ | $0.24^{c}$ | 0.007 | * |
| Rumen passage rate (%/h) | $4.12^{a}$ | $3.39^{b}$ | $3.34^{b}$ | 0.14 | ** |
| Rumen retention time (h) | $24.27^{b}$ | $29.50^{a}$ | $29.94^{a}$ | 0.53 | ** |
| Total retention time (h) | $58.24^{c}$ | $61.23^{b}$ | $64.29^{a}$ | 0.56 | *** |
| Eating (min/d) | $322.78^{a}$ | $305.56^{b}$ | $313.89^{b}$ | 3.321 | * |
| Rumination (min/d) | $379.44^{b}$ | $358.33^{c}$ | $416.67^{a}$ | 2.125 | *** |
| Total chewing activity (min/d) | $702.22^{b}$ | $663.89^{b}$ | $730.56^{a}$ | 3.124 | *** |
| Milk yield (kg/d) | 34.76 | 34.03 | 34.83 | 0.381 | NS |
| Milk fat (%) | $3.34^{b}$ | $3.34^{b}$ | $3.45^{a}$ | 0.021 | ** |
| Milk protein (%) | $3.23^{b}$ | $3.27^{a}$ | $3.27^{a}$ | 0.084 | * |

[1]Means within a row with different subscripts differ ($P < 0.05$). NS, Not significant; $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$.

According to ruminal pH, grinding and pelleting significantly decrease and increased effectiveness of fiber. Results from this experiment shows that based on milk fat assay, grinding did not significantly reduce effectiveness of BP, but pelleting significantly increased effectiveness of BP. In addition, based on total chewing activity, grinding did not significantly reduce physically effectiveness of BP, but pelleting of BP significantly increased physically effectiveness of BP.

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# Enrichment of Cow Milk by Feeding Potassium Iodide

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**Introduction** Iodine is an essential dietary element for mammals, required for the synthesis of the thyroid hormones; thyroxin (T4, 3,5,3',5'-tetraiodothyronine), and its active form T3 (3,5,3'-triiodothyronine) (SCF,2002). Thyroid hormones play a major role in the growth and development of brain and central nervous systems, control of several metabolic processes in body including carbohydrate, fat, protein, vitamin and mineral metabolism (EFSA, 2005). Milk and dairy products are an important source of iodine for human. Iodine concentration of cow milk can be influenced by its concentration in the diet or pasteurization process. A Linear correlation between iodine content of the diet and concentration in cow milk has been reported (Hemken; 1979, Fish & Swenson; 1982, Lysbet et. al, 2003). This study was conducted in order to increase the iodine concentration of cow milk in accordance with human requirements by examining (i) inclusion rate of iodine in the animals diet and (ii) the effect of pasteurization process.

**Materials and methods** Sixteen Holstein cows, with an average daily milk of 32.9 ± 2.4 kg and 189 ± 27 days in milk, were assigned to four treatments in a completely randomized design. The experimental diets were the control, the control diet plus 2.5, 5 and 7.5 mg Potassium Iodide/kg DM. The experiment lasted for 8 weeks. The iodine content of blood, urine, raw and pasteurized milk, weekly and blood thyroidal hormones ($T_3$ and $T_4$), monthly was determined. The dry matter intake, milk production and composition were measured for all treatments. Milk was pasteurized by HTST (High Temperature Short Time) method in order to study the effect of heat stress on iodine concentrations. Data were statistically analyzed by repeated measures procedure using mixed models and the SAS® software package.

**Results** The iodine concentration of blood, urine, raw and pasteurized milk samples was affected (P<0.0001) by dietary levels of potassium iodide (Table 1). No differences (P > 0.05) were observed between treatments for DMI, feed efficiency, milk and FCM (4% fat) production, amount and concentration of milk composition (fat, protein, lactose and solid non fat) and thyroidal hormones. Iodine concentration was reduced (P < 0.05) during the pasteurization process in all treatments (Figure 1). Other studies have noted that the reduction of iodine content due to pasteurization was in the range of 20-33% (Magee et. al, 1968 and Pedriali et. al, 1997). These results show that the common diets fed to dairy cows in Iranian dairy farms will not result in adequate supply of milk iodine for humans but that increasing the iodine level up to 7.5 mg Potassium Iodide/kg diet may provide most of the consumer needs (Table 2).

**Table 1** Iodine concentration of blood, urine, raw and pasteurized milk samples

| Iodine (µg/L) | Treatment (mg/Kg DM) | | | | Probability | SEM |
|---|---|---|---|---|---|---|
| | 0 | 2.5 | 5 | 7.5 | | |
| Urine | 59.5 | 364.2 | 601.7 | 681.9 | <0.0001 | 19.1 |
| Blood | 177.37 | 326.8 | 339.6 | 381.4 | <0.0001 | 14.4 |
| Raw milk | 142.6 | 419.8 | 535.1 | 538.7 | <0.0001 | 35.6 |
| Pasteurized milk | 67.43 | 306.9 | 367.5 | 466.8 | <0.0001 | 28.3 |

**Table 2** Human requirement and rate of iodine supply in basal diet and Treatment 4 (in 200 cc = 1 cup milk)

| Age | Requirement* (µg/day) | Supply (%) | |
|---|---|---|---|
| | | Basal diet | Treatment 4 |
| 0 – 59 months | 90 | 15 | 104 |
| 6 – 12 years | 120 | 11 | 78 |
| Adults | 150 | 9 | 62 |
| Pregnancy | 200 | 7 | 47 |
| Lactation | 200 | 7 | 47 |

* WHO/UNICEF/ICCIDD (2001)

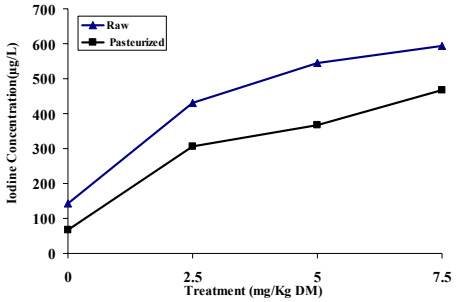


**Figure 1** – Iodine concentration of the raw and pasteurized milk

**Conclusions** Because of the linear increase in iodine concentration in cow milk through feeding increased inclusion rates of Potassium Iodide in the animals diet, increasing iodine concentration through dietary supplementation could supply human requirements and in particular, that of children. This work will provide farmers with guideline to the appropriate for inclusion rates of iodine in dairy cows diets to meet consumers' requirements.

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# Variations in cows' plasma and milk carotenoid concentrations following changes in carotenoid intake level

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**Introduction** Dairy products composition and quality are strongly influenced by the nature of the diet fed to cows, including their composition in micronutrients, among them carotenoids. These compounds are pigments that develop provitamin A and antioxidant functions, take part in the maintenance of cell communication, enhance the immune function, and UV skin and macula protection (Van den Berg et al., 2000). In dairy products, besides to having nutritional and sensorial properties, they have been recognized as tracer compounds indicating animal feeding management (review of Nozière *et al.,* 2006). Factors controlling their concentration in cow's milk must therefore be highlighted. The aim of this study was to determine the variation in plasma and milk carotenoids concentrations when cows shifted from a low-carotenoid diet to diets containing various levels of carotenoids.

**Materials and methods** This study was performed on 32 multiparous Montbéliarde dairy cows in mid-lactation. After a 6 weeks pre-experimental period on a low-carotenoid diet based on hay and concentrates, cows were allocated to 4 homogeneous groups, and thereafter fed for 6 weeks isoenergetic experimental diets based on hay substituted by an experimental feed rich in carotenoids consisting in grass silage and lucerne protein concentrate[1] (75:25). The proportions experimental feed: hay tested were 100:0 (group 1), 66:33 (group 2), and 33:66 (group 3) or 0:100 (group 4). For each group, the daily amount of forage was similar for all animals and constant over the experiment, providing respectively 7.4, 5.4, 3.6 and 1.6 g/d carotenoids (1.63, 1.13, 0.65 and 0.15 g/d ß-carotene). Cows were supplemented with concentrates according to their individual energy and nitrogen requirements, and they received a mineral-vitamin supplement to meet their requirements in vitamins A, D3 and E. Individual milk yield was recorded daily and milk samples were collected twice a week for milk fat determination. Plasma and milk samples were collected on days -1, 5, 8, 14, 21, 28 and 42 related to distribution of the experimental diets, for determination of carotenoids by HPLC. Data were analyzed as repeated measurements using the MIXED procedure of SAS, with group, sampling time and their interaction as fixed effects, and animal as random effect.

**Results** Milk yield and milk fat averaged 18.7 kg/d and 35.1 g/kg, respectively. Fat yield did not differ among groups. Zeaxanthin, lutein, 13-cis-ß-carotene and all-trans-ß-carotene accounted for 3, 10, 9 and 78% of total carotenoids in plasma, and 0, 17, 12 and 71% of total carotenoids in milk, respectively. Plasma and milk carotenoid concentrations were constant over time in group 4, and increased in the other groups ($P<0.001$, Figure 1). In plasma, a plateau was reached from day 28 (group 3), but was not reached at day 42 for groups 1 and 2. In milk, a plateau was reached from day 21 (group 2) or day 28 (groups 1 and 3). Plasma concentration was linearly related to carotenoids intake level and was different between groups from day 5 ($P<0.05$). In contrast, the milk fat carotenoids concentration and the daily yield of carotenoids in milk were similar for groups 1, 2 and 3. They differ from group 1 from day 28 onwards ($P<0.05$)

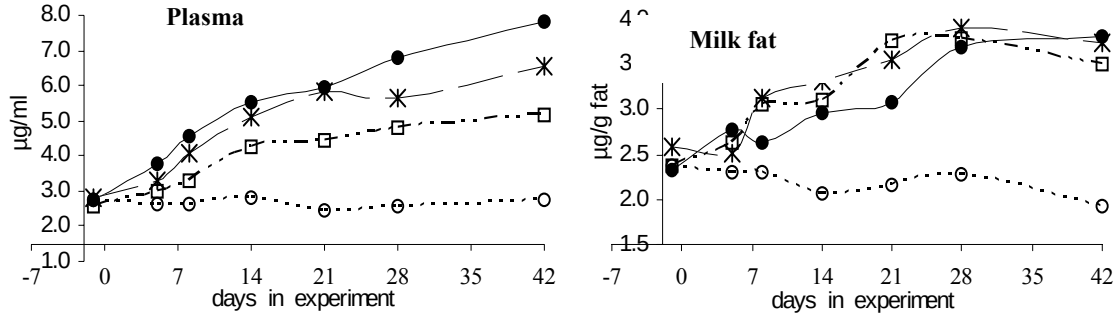


**Figure 1** Variations in plasma and milk fat carotenoids concentrations in cows fed different levels of carotenoids: groups 1 (●); 2 (∗);3 (□);4 (o );

**Conclusion.** The increase in carotenoids concentrations in plasma and milk occurred rapidly when cows shifted from a low- to a high-carotenoid diet. In plasma, the time required to reach the plateau varied according to the carotenoids intake level. Although the plasma concentration linearly increased with the carotenoid intake level, saturation in milk concentrations occurred at high levels of carotenoid intake.

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# Responses of early lactating dairy Holstein cows to diet containing acid treated lucerne silage

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**Introduction** Ensiling of forage rather than drying for hay become more common in many areas because of weather condition, field losses and other risks. Lucerne protein is subjected to extensive degradation during ensiling. Organic and inorganic acids as silage additives could decrease protein degradation of lucerne during ensiling. Cows fed on formic acid treated lucerne silage produced more daily milk and milk per ton of harvested forage. The objective of this study was to determine the effects of diet containing untreated (U) or treated (T) lucerne silage with formic and sulphuric acids on performance of early lactating dairy Holstein cows.

**Materials and methods** Two silages were made according to the previous study (Untreated silage or formic + sulphuric acids treated silage, 15 and 4 ml/kg DM, respectively; Behgar et. al, 2004). After 45 days, silages where opened and included in the basal diet (17% crude protein and 11 MJ/kg DM metabolizable energy) instead of 0.5 of lucerne hay. Nitrogen fractions of silages determined according to Licitra *et al.* (1996) and NDF determined according to van soest *et al*. (1991). The experimental diets fed to 11 multiparous Holstein fresh cows with 19±8 DIM according to completely randomized design for 8 weeks. First week consider as adaptation period. Animals were housed in tie stall, diets were offered (ad lib) as TMR. Animals had free access to water and salt. Feed was offered twice daily and refusals were recorded once daily. Dry matter intake (DMI) and milk production recorded daily. Fresh samples of milk were taken weekly at three consecutive milking and pooled based on production for fat, protein, lactose and solid not fat (SNF) analysis (Milko-tester, Conveyor 4000; Foss Electric). Data were covariatly adjusted for first week and analyzed as repeated measures in time using the mixed procedure of SAS. The statistical model was: $Yijk = \mu + Di + Wj + Ck(i) + Eijk$. Where Yijk = the dependent variable for diet i, week j for cow k in diet i; $\mu$ = the overall mean; Di = effect of diet, i = 1, 2; Wj = effect of week, j = 1, 2, 3, 4, 5, 6; Ck (i) = effect of cow k in diet i, k =1, 2; Eijk = residual.

**Results** The chemical compositions of the silages are shown in Table 1. The effects of diets on milk production and milk composition are shown in Table 2. The diets had no significant effect on DMI, milk production and milk composition. Milk DM, protein and SNF tended to increase by T diet. Time effect on milk fat concentration and SNF was significant ($P<0.01$). There was no interaction between the time and treatments.

**Conclusions** Results of this experiment showed that a mixture of formic and sulphuric acids might use as an additive for lucerne silage. When the mixture of formic acid and sulphuric acid were used, silage pH was decreased and CP increased (Table 1). The numerically increase in milk SNF and DM might be related to the effect of T diet on milk protein. Milk protein increased ($P=0.08$) numerically by T diet. It seems cows fed the T diet had more efficient milk production compare with other cows.

**Table 1** Chemical composition of silages.

| | U | T |
|---|---|---|
| DM (%) | 26.14 | 27.3 |
| pH | 5.38 | 4.5 |
| CP (% DM) | 16.91 | 19.71 |
| TP (% DM) | 10.05 | 11.6 |
| NPN (% DM) | 8.02 | 8.01 |
| NDF (% DM) | 42 | 40 |

U: Untreated and T: Formic acid treated lucerne silage

**Table 2** Least square means of DMI, milk yield and milk composition of early lactating Holstein dairy cows fed diets containing untreated (U) or formic acid + sulphuric acid treated (T) lucerne silage.

| | Treatment Effect | | | | Time Effect | | |
|---|---|---|---|---|---|---|---|
| | U | T | SE | P | L | Q | SE |
| DMI (Kg/day) | 21.07 | 21.57 | 0.99 | 0.64 | 0.24 | 0.21 | 1.33 |
| Milk production (kg/day) | 33.14 | 34.31 | 1.11 | 0.30 | 0.61 | 0.59 | 1.25 |
| Milk Composition | | | | | | | |
| DM (g/Kg) | 107.68 | 110.30 | 1.29 | 0.07 | 0.36 | 0.33 | 1.98 |
| Fat (g/Kg) | 30.47 | 30.55 | 1.23 | 0.95 | <0.01 | <0.01 | 1.89 |
| Protein (g/Kg) | 30.00 | 31.31 | 0.73 | 0.08 | 0.58 | 0.59 | 1.11 |
| Lactose (g/Kg) | 45.49 | 45.41 | 0.45 | 0.85 | 0.89 | 0.4 | 0.7 |
| SNF (g/Kg) | 77.96 | 79.85 | 1.24 | 0.057 | <0.01 | 0.04 | 1.91 |

L**:** Liner effect of time. Q**:** Quadratic effect of time.

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# Effects of substitution barley grain with dried citrus pulp on performance of Holstein dairy cows

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**Introduction** Dried citrus pulp (DCP) as a by-product from juice extraction of citrus fruit can be used as a feedstuff for ruminant animals (Belbaskis and Tsirgogianni, 1996). The DCP is composed of peel, inside portions, and culled fruits of the citrus family (e.g., orange, lemons, and grapefruit). The DCP is a pectin-rich bulky concentrate that is rich source of energy, fibre and calcium, but poor in CP and phosphorous. There is a different of opinion among researchers about the effect of DCP on milk yield, milk composition and blood metabolites components. The main objective of this experiment was to evaluated effects replacement of dried citrus pulp with barley grain on milk yield, milk composition and blood metabolites of dairy cows.

**Materials and methods** Eight lactating Holstein cows ( 70 ± 10 days postpartum, ,weighing 600 ± 20 kg) were randomly allocated to the treatments based on calving date , lactating number and daily milk production in a 4×4 Latin square design. The dietary treatments were as follows: 1) the control diet ( lucerne 15% , barely silage18% , whole cotton seed 8%, maize17%, barley grain 15% , canola meal 12%, cotton seed meal 7%, wheat bran 5%, by Vitamins and minerals supplement 3%), 2, 3 and 4) the control diet plus 5, 10 and 15% of barley grain which were replaced with DCP (W/W). Each experimental period was 21 days including 14 days adaptation period and 7 days collection samples. Milk yield recorded daily and milk sample were taken from each milking times during the last 3 days of each period. Milk samples were subjected to analysis for CP, lactose, fat and SNF. Blood samples were drawn from the jugular vein into evacuated tubes on the last day of each experimental period at about 3-4 h post feeding. The collected serum was frozen at the -20ºC after centrifuging for the future analysis for glucose, urea, cholesterol and triglyceride concentrating. Data were analysed by using procedure GLM, SAS (9.1).

**Results** The dry matter intake (DMI), milk yield and composition are presented in the Table 1. Differences between treatments for DMI and milk yield were not statistically significant (P>0.05). The substitution of barley grain by the DCP at 10 and 15 % levels increased milk fat content compare to the control diet and the difference between these figures was significant (P<0.05). The blood metabolites were not affected by the experimental treatments although the blood cholesterol level intended to increase by addition DCP (Table 1).

**Table 1** Dry matter intake, milk production, and composition and blood metabolites of experimental dairy cow

| | Treatment (citrus pulp level %) | | | | |
|---|---|---|---|---|---|
| Item | 0 | 5 | 10 | 15 | SEM |
| DMI (kg day$^{-1}$) | 20.02 | 20.26 | 19.8 | 20.41 | 1.46 |
| Milk yield (kg day $^{-1}$) | 34.95 | 34.75 | 32.63 | 32.15 | 4.17 |
| Milk composition (%) | | | | | |
| Protein | 3.13 | 3.12 | 3.10 | 3.13 | 0.14 |
| Lactose | 4.66 | 4.69 | 4.65 | 4.63 | 0.22 |
| Fat | 2.88$^{b}$ | 2.95$^{ab}$ | 3.14$^{a}$ | 3.40$^{a}$ | 0.44 |
| SNF | 8.46 | 8.50 | 8.46 | 8.42 | 0.34 |
| Blood metabolites (mg/dl) | | | | | |
| Glucose | 50.41 | 52.13 | 50.77 | 46.6 | 2.92 |
| BUN | 13.33 | 14.16 | 12.06 | 11.41 | 2.78 |
| Cholesterol | 213.5 | 211.25 | 234.00 | 241.37 | 11.28 |

Means in row with different superscript letter are different (P<0.05)

**Conclusion** The obtained results indicated that substitution of barley grain by DCP led to higher level of milk fat content. Milk fat content is an economical factor. This result is in agreement with the finding of Economides (1974) and Zervas (1994). The addition of DCP at 15% level (dry matter basis) to the dairy cow ration will decrease the cost of milk production without any adverse effects on animals. This by- product can be regarded as a source of effective fibre for ruminant animal. However, more experiments are needed for confirmation of these finding and suggestion.

**Acknowledgement** The financial support of Excellence Center for Animal Science, Ferdowsi University of Mashhad is greatly appreciated.

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# Effects of dried and ensiled tomato pomace on dry matter intake, milk yield and composition of dairy cows in Iran

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**Introduction** In Khorasan province of Iran about 1 million tonnes of tomatoes are grown annually. They are either directly sold to the customers as vegetable (200,000 tonnes) or further processed to products such as paste, ketchup, sauces etc. After the juice is extracted from fresh tomatoes, a residue primarily consisting of water, tomato seeds and peels is left. Tomato processing residues which is called tomato pulp accounts about one fifth of fresh weight or 160,000 tonnes in the area (ministry of Jahad- Agriculture, 2004). Fresh tomato pulp becomes sour and mould rapidly because it is traditionally processed during summer time and has high moisture content. Consequently, it is advisable to be ensiled or dried. Although it is commonly fed to ruminants but little data is available on its effect on milk yield, milk composition and dry matter intake in dairy cows.

**Materials and methods** Nine lactating Holstein dairy cows with mean body weight of 600 kg and 70 ± 10 days in milk were randomly allocated to the treatments based on calving date, lactating number and daily milk production in a 3×3 Latin square design. There were three cows per each treatment and treatment of cows were changed periodically. The dietary treatments were as follows: 1) the control diet, 2 and 3) diets containing 10% sun dried and ensiled tomato pomace. Each experimental period consisted 21 days including 14 days adaptation period and 7 days for collection samples. Milk yield recorded daily and milk sample were taken from each milking times during the last 3 days of each experimental period. Milk samples were subjected to analysis for CP, lactose, fat and SNF. Dry matter intake of each treatment was measured during last 7 day of each experimental period. . Data where analysed by using proc GLM, SAS (9.1).

**Results** The dry matter intake (DMI), milk yield and composition are presented in Table 1. Differences between treatments for DMI and milk yield were not statistically significant (P>0.05). The treatments containing 10% tomato pomace numerically decreased milk fat content compare to the control diet (P>0.05). Dry matter intake was not significantly different between the treatments. The similar results have been reported elsewhere (Weiss, 1997).

**Table1.** Dry matter intake, milk production, and composition

| Item | Treatment (diet) | | | SEM |
|---|---|---|---|---|
| | The control | Dried tomato pomace | Ensiled tomato pomace | |
| DMI (kg day$^{-1}$) | 25.64 | 26.50 | 24.77 | 0.65 |
| Milk yield (kg day $^{-1}$) | 41.33 | 41.12 | 41.31 | 0.357 |
| Milk composition | | | | |
| Protein (%) | 3.09 | 3.14 | 3.11 | 0.024 |
| Lactose (%) | 4.63 | 4.72 | 4.69 | 0.156 |
| Fat (%) | 2.86 | 2.69 | 2.64 | 0.17 |
| SNF (%) | 8.43 | 8.56 | 8.50 | 0.067 |

**Conclusions** The obtained results indicated that the addition of dried and ensiled tomato pomace at a level 10% to the dairy cow diets could decrease the cost of milk production without any adverse effects on the animals. This by- product can be regarded as a cheap source of protein for ruminant animals (Valizadeh and et. al 2006). However, more experiments are needed for inclusion the higher level of this by-product.

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# Effects of birth weight and sex on some economic traits in Brown Swiss calves

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**Introduction** From birth to weaning calves tolerate most stress include metabolic stress and parturition and at new environment obtained nutrients from milk instead of mother blood. In order to successful production of calves, a set of nutritional and environmental management is needed. Calves mortality from birth to weaning is too much and cost effective for dairy industry. Instead, there is low mortality of heifers from weaning to parturition (1). Brown Swiss breed has low population in respect to Holstein in Iran. Low data is available about Brown Swiss population especially Brown Swiss calves. This study conducted to determine some information about Brown Swiss calves from birth to weaning and factors affecting economic traits at this period.

**Materials and methods** In order to conduct this study all parturitions at a Brown Swiss (Animal Science Department Farms, Education Center of Jihad-e Agriculture) herd in an open shed system was recorded in 1999 to 2005. 544 cows were calved between these two date. Calves were separated 1 hour after parturition and housed at single boxes up to weaning. Records were included: birth weight (BW), birth date, weaning weight (WW), weaning date and sex of calf. Body weight gain before weaning (BWG) and preweaning period (PP) were calculated using these data. Calves were grouped according to birth weight 1- less than 40 Kg 2- less or equal to 45 and more or equal to 40 Kg 3- more than 45 Kg. Data analyzed in a completely randomized design using SAS 6.12. Means were compared using Duncan test ($P<0.05$).

**Results** As can be seen in table 1 weaning weight had been increased significantly with increase in birth weight ($P<0.01$). Preweaning period didn't influenced by birth weight. Daily weight gain increased significantly ($P<0.07$) so increasing weight at birth had lead to more growth rate before weaning. Table 2 shows that birth weight is higher in males than females in Brown Swiss calves just like Holsteins ($P<0.01$). Weaning weight is higher in female than male calves ($P<0.01$), which is a result of longer preweaning period in females ($P<0.01$).

**Table 1** Effect of birth weight on some economic parameters in Brown Swiss calves

| Items | Groups based on Birth Weight (Kg) | | | SEM |
|---|---|---|---|---|
| | <40 | ≥ 40 and ≤ 45 | >45 | |
| WW[1] (Kg) | 54.02 [a] | 63.26 [b] | 67.18 [c] | 0.513 |
| PP[2] | 56.19 | 55.96 | 54.12 | 0.516 |
| BWG[3] (Kg) | 336.63 | 379.44 | 340.45 | 8.836 |

- Means with different characters are significantly different
1- Weaning Weight 2- Preweaning Period 3- Body Weight Gain before weaning

**Table 2** Investigating some traits in two sex of Brown Swiss calves

| Items | Female | Male | SEM |
|---|---|---|---|
| BW[1] (Kg) | 41.04 [a] | 42.95 [b] | 0.330 |
| WW[2] (Kg) | 63.40 [a] | 60.08 [b] | 0.582 |
| PP[3] | 59.15 [a] | 52.45 [b] | 0.482 |
| BWG[4] (Kg) | 385.99 [a] | 330.35 [b] | 8.743 |

- Means with different characters are significantly different
1- Birth Weight 2-Weaning Weight 3- Preweaning Period 4- Body Weight Gain before weaning

**Conclusions** Calves have more potential to get higher weights at weaning when got birth at higher weights maybe because of more DMI based on larger digestive tract and lead to sooner extension of rumen and better utilization of nutrients. At this herd calves were weaned at a pre-determined age so the preweaning period wasn't influenced by birth weight. Although it's obvious today that weaning must take place according to DMI of calves based on birth weight (2). It seems that more WW of females than males is due to higher milk intake of females than males (59.15 vs. 52.45) ($P<0.01$) and had been contributed to higher BWG in females vs. males ($P<0.01$).

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# Effect of weaning twice daily milk fed calves at low or high levels of concentrate intake on performance to 12 weeks old

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**Introduction** Artificial rearing is a common practice for rearing calves from the dairy herd destined for beef production. In commercial practice calves are typically weaned from 5 to 9 weeks old. There are four criteria that can be used to determine weaning time: age, compound feed intake, liveweight and milk price. Late weaning systems are based on the theory of giving the calf the best possible start in life, but are costly with high milk intakes (Davis and Drackley, 1998). Hence emphasis is usually placed on early weaning of the calf and encouraging concentrate intake. A number of factors can affect concentrate intake including quality and quantity of milk fed, size of the calf and concentrate texture. Recommendations are to typically wean calves when eating 1kg concentrate per day. The objective of this experiment was to evaluate the effect of weaning twice daily milk fed calves on either a low (0.75kg) or high (1.25kg) concentrate intake on performance to 12 weeks old.

**Materials and methods** Thirty-six Holstein and Limousin cross Holstein bull and heifer calves were assigned in a randomised block designed experiment. The calves started the trial at 1 day of age and were individually penned on straw. From days 1 to 4 they were fed colostrum. Calves were fed a whey based milk replacer (DM 967 g/kg, 222g crude protein/kg, 182 g/kg Ether Extract [Wynngold Calf Milk Replacer, Wynnstay plc]) mixed at 125g per litre of water at 40$^{o}$C twice per day. From 5 to 7 days the milk was fed at 4 litres per day and from day 8 to weaning milk was fed at 5 litres per calf per day. From day 4 the calves received *ad libitum* concentrates (DM 866 g/kg; 209g crude protein/kg DM, 251g NDF/kg DM, 37g ether extract/kg DM, and 247g starch/kg DM [Start 'n' Wean pellets, Wynnstay plc]) plus water. The calves were weaned when eating either 0.75kg (Low) or 1.25kg (High) of concentrates per day.The calves were moved into group pens at weaning. The data were analysed by ANOVA with calves blocked according to sex and breed.

**Results** The calves weaned at the high level of concentrate intake recorded significantly higher DLWG's to weaning ($P<0.05$), weaning to 12 weeks of age ($P<0.001$), weaning weights ($P<0.001$) and coat bloom score ($P<0.05$). They were weaned 10.1 days later at approximately 7.5 weeks old and recorded significantly higher milk and concentrate intakes ($P<0.001$). The calves weaned at the low level of concentrate intake were approximately 6 weeks old at weaning. There were no significant differences in DLWG from birth to 12 weeks old and 12 week weights. Milk and concentrate feed intakes were significantly higher from birth to weaning with the high treatment however total concentrate intake from birth to 12 weeks was highest with the low treatment. There were no differences in the health or condition of the calves.

**Table 1** Effect of concentrate intake on liveweight (kg),weaning age (days) and coat bloom score

| | Low | High | s.e.d | Sig |
|---|---|---|---|---|
| Birth weight | 45.9 | 46.7 | 2.09 | NS |
| Weaning weight | 59.8 | 67.1 | 2.05 | *** |
| Weaning age | 42.5 | 52.6 | 2.24 | *** |
| 12 week weight | 101.4 | 105.7 | 4.12 | NS |
| Coat bloom score* | 2.18 | 2,56 | 0.148 | * |

* Coat bloom score scale of 1 = dull, 2 = normal, 3 = shiny.

**Table 2** Effect of concentrate intake on DLWG (kg/day)

| | Low | High | s.e.d | Sig |
|---|---|---|---|---|
| Birth – weaning | 0.33 | 0.38 | 0.031 | * |
| Weaning - 12 weeks | 1.00 | 1.23 | 0.064 | *** |
| Birth - 12 weeks | 0.66 | 0.70 | 0.071 | NS |

**Table 3** Feed intakes (kg/head)

| | Low | High | s.e.d | Sig |
|---|---|---|---|---|
| Milk replacer | 26.5 | 32.9 | 2.01 | *** |
| Conc intake (birth –weaning) | 8.7 | 15.1 | 1.139 | *** |
| Conc intake (birth – 12 weeks) | 121.5 | 113.7 | | |

Based on the prices prevailing at the time of the study with milk replacer costing £1,000/t and concentrates costing £170/t, the total feed costs per calf were £52.23 and £47.15, and the feed costs per kg LWG were 88.5 and 85p for the high and low concentrate weaning intake treatments respectively.

**Conclusions** Weaning calves when eating a high level of concentrates significantly increases weaning age and weight, however weaning calves at a low level of concentrate intake has no significant effect on performance to 12 weeks of age. Calves weaned at the low level of concentrate intake had reduced calf rearing costs. The calves weaned at a high level of concentrate intake recorded a significant improvement in calf coat bloom score. This could have a marked improvement in calf value for producers rearing and selling calves at 12 weeks of age.

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# The impact of farmer and veterinary surgeon concordance on the effectiveness of a lameness control programme for primiparous dairy heifers

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**Introduction** Lameness in dairy cattle is a multifactorial problem involving a complex interaction of stockperson, environmental and animal-related hazards. With over 100 potential hazards for lameness, and a multiplicity of control measures arising from each of these hazards, decision making for intervention programmes can be complex. Furthermore, control of foot lesions such as digital dermatitis and claw horn disease may require a detailed understanding of veterinary pathogenesis and epidemiology. Therefore, in order for lameness control programmes to be effective, veterinary involvement may be necessary. This paper examines effect of vet and farmer attitude towards a lameness control programme for primiparous dairy heifers on lameness prevalence.

**Materials and methods** Thirty farmers and their veterinary surgeons were enrolled onto a 12-month lameness programme consisting of standard elements including diagnostic assessments, risk scoring, intervention planning, monitoring and evaluation. Lameness in cohorts of primiparous heifers was assessed before and after intervention, using a 6-point locomotion score. Score 0 was classified as sound (score 0), scores 1 to 5 were termed unsound. Locomotion scores 4 and 5 were classified as *severely lame*. At re-evaluation, the farmers and local vets involved with the intervention programme were assigned a score based on their attitude towards the lameness control programme, assessed using programme concordance. Score 1 indicated non-withdrawal but no signs of active participation in the project; score 2 indicated participation with one or two elements of the lameness control programme; score 3 was given if there were obvious signs of participation for most of the elements of the programme and score 4 was given for completion of all the assigned activities. Half scores were used. Spearman rank correlations were used to assess the relationship between attitude scores and change in heifer unsoundness prevalence.

**Results** Twenty-eight farms completed the programme, with 2 farms withdrawing due to herd dispersal. Within the intervention group, vet and farmer scores for concordance with the lameness control programme ranged between 1 and 3, with a median score of 2. Two vets failed to initiate the lameness control programme within the 12 month intervention period, resulting in a score 1 for both vet and farmer. There was no correlation between concordance scores for farmer and their vet. Farmer scores were bimodal, with scores 1.5 and 2.5 being most common (n=9 in both), while score 1.5 was most common for vets (n=11). Overall lameness prevalence increased during the intervention period. Three farms achieved a reduction in unsoundness prevalence and 11 farms reduced severe lameness prevalence. Change in *unsoundness* prevalence was not significantly correlated with farmer concordance score ($r= -0.306$, $p=0.114$). However, there was a significant negative correlation between veterinary surgeon concordance and *unsoundness* prevalence ($r= -0.429$, $p=0.023$). When attitude scores for local vet and farmer were considered as an interaction, there was a highly significant relationship between *vet*farmer attitude* and change in *unsoundness* prevalence ($r= -0.498$, $p=0.007$). Change in severely lame prevalence was negatively correlated with farmer concordance ($r=-0.385$, $p=0.43$) but not vet*farmer attitude ($r=-0.308$, $p=0.111$) or vet concordance ($r=-0.154$, $p=0.434$). Vet, but not farmer, concordance was highly significantly correlated with lameness unsoundness at the start of the intervention period ($r=0.501$, $p=0.007$). Concordance was not related to severe lameness prevalence at the start of the intervention period.

**Conclusions** For lameness control programmes to have a measurable impact on lameness prevention (as indicated by unsoundness prevalence), programme concordance by the veterinary surgeon is important. Programme concordance by both the farmer and veterinary surgeon would appear to be necessary to achieve the best results. For alleviation of severe lameness, farmer concordance would appear to be more important.

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# Analysing the risk of a bovine brucellosis epidemic in Great Britain using the cattle tracing scheme: have we just been lucky so far?

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**Introduction** Bovine Brucellosis is a widespread, economically devastating and highly infectious zoonosis caused by *Brucella abortus*. In cattle it causes premature abortion around five to seven months into the normal nine month gestation and the disease can be transferred to humans through milk. Great Britain (GB) has been "Officially Brucellosis Free" (OBF) since 1991 and it is in the country's best interest to maintain this status. There have been three reintroductions of the disease since 2003, the most recent in a beef herd in Cornwall 2004 (DEFRA, 2004). Such outbreaks threaten the UK's OBF status. By identifying epidemiological risk factors and using data from the Cattle Tracing Scheme for GB (CTS) we examined the spatial and temporal patterns of births for both the whole of GB and the South West region in particular and used this information to identify risk periods due to cattle births. Then cattle movements originating from, or ending in Cornwall (the location of the last outbreak) were identified from the CTS database. A subset of 57 000 high-risk, potentially infectious, moves were identified and examined to establish the potential spatial spread of the disease from these movements.

**Materials and methods** Cattle Tracing System data was sourced through DEFRA's RADAR unit (Rapid Analysis and Detection of Animal Related Risks). The CTS defines movements as including: births, deaths and movements -on and movements -off specified locations, with dates and individual identification. Analysis was performed in the Microsoft Access 2003 database. Each field was given an individual name and source table identified by its initials to allow traceability as the merged files became more complex. Time of parturition has been identified as a key risk factor for the spread of Brucellosis. Therefore, data were extracted to characterise the temporal and spatial birth patterns for Great Britain as a whole, for a subset relating to the area of interest: the south west of England, (the counties of Cornwall, Devon, Dorset, Avon, Somerset and the Isles of Scilly: -Figure 1). This was further divided into DEFRA-designated breed codes and breed purpose codes to ascertain any differences. DEFRA-designated beef breeds were then focussed on to investigate potential spatial spread of disease by identifying potentially high-risk movements from all those movements that originated from, or ended in Cornwall. By using georeference data it was possible to create a visual representation of these individual movements illustrating spatial distributions of the potentially infectious movements at different probability values (Fig 2).

**Results** Risk factors identified: 1) Risk of within-herd spread constantly present due to temporal distribution of births peaking between October and May for beef breeds. 2) Highest potential risk movements for spatial spread are female beef cattle that move from farm to farm with 54,000 moves of this type occurring in Cornwall in 2004, giving opportunity for herd –to –herd –transmission. 3) Large farms have a higher potential risk, although this looked at individual moves rather than batches. The mean number of days per annum that any farm had multiple off movements to the same location was four. 5) Risk from imported animals was negligible over this period.

**Figure 1** Births per month in the SW by region

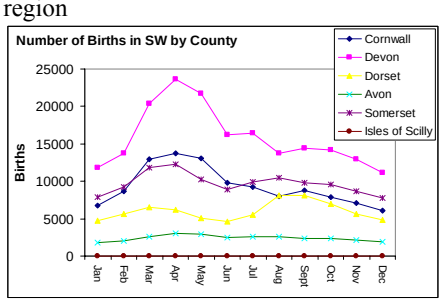
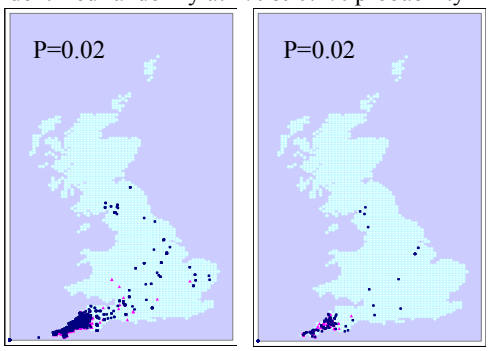



**Figure 2** Potentially infective moves identified randomly at 2% & 0.2% probability




**Conclusions** This study shows that there is a continuing risk to the UK from bovine Brucellosis and there is significant variation in the potential for within-herd transmission. Long-distance cattle are movements out of Cornwall lead to many parts of GB with a real risk of widespread transmission. However, the risk of an initial outbreak in Cornwall is no higher than elsewhere in the country, but as a net exporter of cattle, should an outbreak occur the risk of onward transmission is probably higher than average. This work illustrates the potential for the CTS cattle movement data to be used to investigate the risk from bovine Brucellosis and other bovine disease. Furthermore, it indicates that while the more stringent UK Brucellosis monitoring policy provides adequate surveillance to identify outbreaks in a timely manner, suggesting that luck has played little part in preventing an epidemic. However, this may not remain the case if we were to revert to the EU monitoring policy that advocates testing once every five years rather than the UK's current two.

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# Prevalence of bovine tuberculosis among Fulani cattle in the Federal Capital Territory (FCT), Abuja-Nigeria

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**Introduction** The re-emergence of tuberculosis (TB) has been observed in both developing and developed countries in recent years. Tuberculosis, a major public health and zoonotic problem, is responsible for 2 to 3 million human deaths annually (WHO 2003) and also causes great economic loss in the animal industry. Tuberculosis has been declared a global emergency by WHO in 1993; the first to be declared as such. Nigeria with a population of over 120 million people and cattle population of about 19.8 million has been ranked 4$^{th}$ among the world's 22 countries with a high TB burden. The objective of this study was to determine the prevalence of bovine tuberculosis among Fulani cattle, which are the main source of milk and milk products to the public. The culture of consuming raw milk as a local delicacy known as 'fura da nono' among Nigerians especially in the study area informed this study.

**Materials and methods** A total of 336 lactating cows whose milk was destined for human consumption were tested for TB with Purified Protein Derivative (PPD) using the single comparative intradermal tuberculin test (SCITT). Skin thickness was measured before and 72hr after injecting tuberculin; and the reading interpreted using standard technique. Cows from 16 different Fulani pastoralist herds were tested and both nomadic and the semi-nomadic herds were included in this study. Prevalence rates of TB among the herds was determined and also compared in the two husbandry systems using Chi-square analysis.

**Results** Of the 336 cows tested, 54 (16%) were positive for tuberculin, 22 (7%) had inconclusive/doubtful result while 250 (74%) tested negative. Out of the 336 cows tested 200 were from the nomadic herds and 34 (17%) of them were found to be positive. One hundred and thirty six were from the semi-nomadic system; out of which 20 (14.7%) cows tested positive. The prevalence rate of TB in the nomadic and the semi-nomadic herd did not show any significant variation ($P<0.05$).

| Group | No tested | Positive reactors | Negative reactors | Inconclusive |
|---|---|---|---|---|
| FCT (All) | 336 | 54 (16%) | 250 (74%) | 22 (7%) |
| Nomadic | 200 | 34 (17%) | 154 (77%) | 12 (6%) |
| Semi-Nomadic | 136 | 20 (15%) | 106 (78%) | 10 (7%) |

**Figure 1** Result of tuberculin test in Cows

**Conclusions** The data reveals that there is high prevalence of bovine tuberculosis in the FCT, Abuja, which is similar to other studies conducted in other parts of the country (0.2-14.5%), (Du-Sai and Abdullahi 1994; Cadmus *et al*., 2004). This might suggest the existence of foci of infection in the country and may be attributed to management practices and also lack of control measures in place. This high level of infection among lactating cows may have contributed to the high incidence of TB observed in humans especially in HIV patients whose immune system is compromised. This data is of great public health importance and calls for prompt control measures to be put in place.

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# Nutritive value index of treated wheat straw with Pleurotus fungi fed to cow

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**Introduction** Biological de-lignification of straw by white-rot fungi seems a promising way of improving its nutritive value. The bio-conversion of lignocellulosic materials is circumscribed to the group of white-rot fungi, of which some species of *Pleurotus* are capable of producing upgraded spent-straws as ruminant feed (Fazaeli *et al*., 2004). Treating of cereal straw with white-rot fungi as animal feed was studied by several workers (Gupta *et al.,* 1993; Zadrazil, 1997). However, most of the trials were conducted at *in vitro* stage and used cell wall degradation and *in vitro* digestibility as an index to evaluate the biological treatments. This experiment was conducted to study the effect of fungal treatment on the voluntary intake, *in vivo* digestibility and nutritive value index of wheat straw obtained from short-term and long-term solid state fermentation (SSF).

**Materials and methods** Wheat straw was chopped into 5-10 cm length and soaked in water for 24h, in a steel water pool, then it was pasteurised at 75±5ºC for one hour. The straw was inoculated with wheat grain spawns of *Pleurotus Florida* at a rate of 3.5-kg spawn per 100-kg straw (fresh weight basis), packed in the plastic bags and incubated in a fermentation room at 25±5ºC and 65±5 relative humidity. After 18 days of spawning and before fruiting body formation, half of the bags were removed from the fermentation room and dried under the sun. For the remainder of the bags, SSF was allowed for seven weeks during which the mushroom was harvested two times, and then they were removed from the fermentation room, dried and used for the experiment. In a completely randomised design, the *in vivo* digestibility and voluntary intake were determined in 12 steers when the treatments were: 1) untreated wheat straw (UWS), 2) fungal treated wheat straw before formation of mushroom (FTWS) and 3) fungal treated wheat straw after harvesting of mushroom i.e. spent wheat straw (SPWS). The dry treated and untreated straws were chopped and fed to the steers *ad libitum* in addition with 500 gram concentrate supplement per animal, composed of ground barley, wheat bran, cotton seed meal and mineral supplement. Data were statistically analysed, according to the compeletely randomized design, using of SAS GML procedure.

**Results** Total tract digestibility of dry mater (DM) and organic mater (OM) were significantly ($P<0.05$) higher in FTWS than the UWS and SPWS (Table 1). When the mushroom was harvested, the digestibility was reduced in the residual straw, in comparison to the FTWS, although this reduction was not statistically significant. Meanwhile, the digestibility of SPWS was numerically higher that the UWS however the difference was not statistically significant. Daily consumption of DM and OM (as kg/d, percent of body weight or g/kg $BW^{0.75}$) were significantly ($p<0.05$) increased when the cows received FTWS but the intake of SPWS was reduced and were similar to that of the UWS. The digestible dry mater intake (DDMI) and digestible organic mater intake (DOMI) were also significantly ($p<0.05$) different among the treatments (Table 1). The significantly ($p<0.05$) highest amount of DDMI or DOMI were obtained in cows fed FTWS and the lowest were obtained in those fed UWS however no significant differences were observed between UWS and SPWS. Similar status where observed when DDMI and DOMI were expressed as g/kg $BW^{0.75}$.

**Table 1** Effect of treatments on digestibility and nutrients intake

| Treatment | Digestibility (g/kg) | | Intake (kg/d) | | Intake (% BW) | | Intake (g/kg$BW^{0.75}$) | | Intake (kg/d) | | Intake (g/kg$BW^{0.75}$) | | NVI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DM | OM | DM | OM | DM | OM | DM | OM | DDM | DOM | DDM | DOM | |
| UWS | $348^{b}$ | $350^{b}$ | $4.46^{b}$ | $4.14^{b}$ | $1.49^{b}$ | $1.38^{b}$ | $63^{b}$ | $58^{b}$ | $1.55^{b}$ | $1.44^{b}$ | $21.5^{b}$ | $20.0^{b}$ | $100^{b}$ |
| FTWS | $452^{a}$ | $448^{a}$ | $5.44^{b}$ | $4.90^{a}$ | $1.81^{a}$ | $1.63^{a}$ | $77^{a}$ | $68^{a}$ | $2.40^{a}$ | $2.20^{a}$ | $33.3^{a}$ | $30.5^{a}$ | $153^{a}$ |
| SPWS | $410^{ab}$ | $415^{ab}$ | $4.28^{b}$ | $3.80^{b}$ | $1.43^{b}$ | $1.27^{b}$ | $59^{b}$ | $53^{b}$ | $1.80^{b}$ | $1.62^{b}$ | $24.4^{b}$ | $22.5^{b}$ | $112^{b}$ |
| SEM | 0.25 | 0.70 | 0.2 | 0.2 | 0.11 | 0.09 | 2.8 | 2.5 | 0.12 | 0.10 | 1.10 | 1.10 | 15.6 |

Means with the different superscripts within a column are significantly ($P<0.05$) different.
UWS = untreated wheat straw, FTWS = Fungal treated wheat straw before mushroom formation
SPWS = Fungal treated wheat straw, after harvesting of mushroom. SEM = Standard error of means.
NVI = Nutritive value index = Relative intake × digestibility coefficient.
Relative intake = Amount of intake from treatment/amount of intake from control.

**Conclusion** In conclusion, treatment of wheat straw with *Pleurotus* fungi resulted in an increasing of *in vivo* digestibility, voluntary intake and nutritive value index, but the straw obtained after mushroom harvesting as spent wheat straw did not influenced these parameters significantly as observed in the case of untreated straw when fed to steers. It appears that the changes in nutritive value of straw may be related to the duration of fermentation straw by fungi and stage of harvesting.

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# Effects of whole crop canola silage on Holstein dairy cow performance in early lactation

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**Introduction** It is clear that providing sufficient fiber in dairy cattle diets has most positive effects on improvement of rumen ecosystem and feed intake efficiency. In semiarid countries such as Iran providing forage requirement of cattle is limited. So it is better to use plants with low water requirement, high growth rate and high forage yield per hectare. 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 many areas of Iran. In addition to its low water requirements and high growth rate, its cultivation season is well matched with environmental condition in Iran. The main objective of this study was to evaluate effect of replacing maize silage with canola silage (as dry matter %) on Holstein dairy cow performance.

**Material and methods** Nine multiparious Holstein cows in early lactation were arranged in 3×3 Latin square design in three periods. Each period consisted of 18 days. The first 13 days were for adaptation and final 5 days were for data collection. The treatments were substitution of maize silage with canola silage (CS) at three levels of 0, 5 and 10% (dry matter) of daily TMR. Canola forage was ensilaged after than harvested at early pudding stage. CS nutrient composition value for DM, OM, CP, EE, NDF, and ADF was respectively 19.5, 90.06, 16.57, 4.8, 50.3, and 38 % of canola silage DM. Chemical composition of diets is shown in Table 1. Diets were fed twice daily to allow ad libitum intake and orts were weighted once daily during days 13 to 18 of each period. Milk production was recorded at the last 5 d of each period and the milk from the two last days of each period was used for milk composition analysis. Rumen fluid was obtained via the ruminal cannula at 3 h after first daily feeding by passage of a hand-capped plastic jar (250 ml) through the fiber mat and collection of fluid from the ventral rumen. Samples were strained through two layers of cheesecloth for pH determination and NH3-N concentration on d 18 of each period. Data were analyzed using the general linear model procedure of SAS (2000).

**Results** The results of this study are shown in Table 2. Replacing maize silage with CS had significant effect on DMI ($p< 0.05$). Except than milk crude protein, diet had no effect on milk yield and composition. Although there were no significant differences in Rumen pH and $NH_3$-N concentrations among groups but their values were increased numerically by replacing maize silage with CS.

**Table 1** Chemical composition of diets

| | Substitution Levels % of TMR | | |
|---|---|---|---|
| Item | 0 | 5 | 10 |
| DM g/Kg | 746.3 | 694.4 | 649.3 |
| NEL Mcal/kg | 1.60 | 1.62 | 1.65 |
| CP g/Kg | 173.0 | 177.0 | 182.0 |
| RDP g/Kg Crude protein | 87.0 | 91.0 | 96.0 |
| RUP g/Kg Crude protein | 85.0 | 86.0 | 86.0 |
| NDF g/Kg | 332.0 | 336.0 | 339.0 |
| ADF g/Kg | 210.0 | 216.0 | 221.0 |

**Table 2** The effect of whole crop canola silage on Holstein dairy cow performance

| | Substitution Levels % of TMR | | | |
|---|---|---|---|---|
| Item | 0 | 5 | 10 | SEM |
| DMI kg/d | 21.60[1a] | 20.9[ab] | 20.38[b] | 0.344 |
| Milk yield kg/d | 36.48 | 35.64 | 36.04 | 0.425 |
| FCM 4 % kg/d | 32.16 | 31.59 | 32.25 | 0.634 |
| Milk Fat g/Kg | 32.1 | 32.4 | 32.9 | 0.083 |
| Milk protein g/Kg | 30.31[a] | 28.78[b] | 30.07[ab] | 0.044 |
| Milk lactose g/Kg | 47.5 | 47.7 | 47.7 | 0.053 |
| Milk SNF g/Kg | 85.2 | 84.7 | 85.1 | 0.08 |
| Rumen fluid pH | 6.43 | 6.55 | 6.56 | 0.076 |
| $NH_3$-N (mg/dl Rumen fluid ) | 20.97 | 20.99 | 22.27 | 2.22 |

1. Data with various alphabets are significantly different ($p< 0.05$ )

**Conclusion** The results of this study indicated that replacing maize silage with CS caused to decrease DMI significantly without any significant effect on milk yield and its constituents except than milk protein content. So it is better not use high level of CS in dairy cattle diets.

**Acknowledgement** The authors wish to acknowledge for funding and technical supporting from Ferdowsi University of Mashhad and Centre of Excellence for Animal Science

**Reference**Lancaster, L. L. and Hunt, C**.** W. 1990. Effects of rapeseed silage variety and dietary level on digestion and growth performance of beef steers. *J. Animal Sci*. **68:** 3812-3820

# Forage intake, milk yield and composition of cows in a silvopastoral system with and without access to the forage tree and energy supplementation during the rainy season

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**Introduction** The improvement in animal performance obtained in silvopastoral systems has been explained either by their ingestion of a higher quality grass (Hernandez et al., 2001, Iglesias, 1998) resulting from the inclusion of a legume in the system which provide N for grass growth or due to the intake of the legume itself, which in turn is also of higher quality than grass. During the rainy season, animals might be able to increase selectivity and intake due to the higher availability of biomass hence possibly reducing the advantage of including a legume in the system. The objective of the present work was to discriminate in a silvopastoral system the effect of improved grass quality and legume intake on dual purpose lactating cows milk yield and composition.

**Material and methods** Eight crossbred cows (*Bos taurus* x *B. indicus*) with 459 ± 47kg LW were used in a latin rectangle design. Two cows were allocated to each treatment during each one of the four 15 d experimental period (10 d adaptation and 5 d sampling). Treatments were. Grass= Guinea grass (*Panicum maximum*) monoculture. SP-L= silvopastoral system (grass + leucaena) but cows did not have access to Leucaena fodder (it was harvested before the cows went into the paddock), SP= silvopastoral system (cows had access to Leucaena fodder), SP+M= silvopastoral system + energy supplementation (maize grain, 0.5% LW). Cows were kept in a 42 d rotational grazing system with 1 d occupation period. Paddock size was adjusted to provide 28 kg DM.animal $d^{-1}$. Intake was estimated using alkanes (Dove and Mayes, 2003). Cows were milked once a day in the morning and during the sampling period 4 I.U. oxytocin was used to ensure complete milk extraction. Preplanned orthogonal contrast of interest were, C1= Grass vs SP-L, C2= SP-L vs SP and C3= SP vs SP+M.

**Results** No major changes were observed in the quality of the grass in the silvopastoral systems as compared with the grass monoculture except for a small increment (7-10%) on its CP content (Table 1). Improvement of grass quality (C1) or inclusion of higher quality feeds (C2 and C3) alone can not explain the improvement in animal performance (Table 2). Total DMI was not affected but the proportions of the dietary component changed.

**Conclusion** It is the cumulative effect of increased DMI and higher diet quality from Grass to SP+M which might explain the observed effects. Alternatively, it is also possible that higher availability and quality of grass during the rainy season allowed animals to have a better diet selection and hence diminishing the effect of including leucaena in the system.

**Table 1** Chemical composition (g.kg $DM^{-1}$) of the grass in the different grazing system and of L. leucocephala fodder.

| | Grass | GSP-L | GSP | L |
|---|---|---|---|---|
| OM | 879 | 886 | 886 | 933 |
| CP | 98 | 105 | 108 | 24.3 |
| NDF | 688 | 680 | 694 | 461 |
| ADF | 415 | 401 | 414 | 258 |
| Lignin | 71 | 67 | 84 | 96 |
| Ca | 8.1 | 5.8 | 7.1 | 9.0 |
| P | 1.3 | 1.3 | 1.6 | 2.2 |

Grass= grass only, GSP= grass in the silvopastoral system GSP-L= GSP without access to leucaena fodder, L= L. leucocephala fodder

**Acknowledgement** The work was funded by project Conacyt-Sagarpa-cofupro-2004-110. V. Valdivia received a scholarship from SRE-Mexico to undergo PhD studies.

**Table 2** Dry matter intake (DMI, kg.$d^{-1}$), milk yield (kg.$d^{-1}$) and composition (g.kg $Milk^{-1}$, except milk urea mg.$dL^{-1}$) in cows under different system.

| | Grass | SP-L | SP | SP+M | SEM | C1 | C2 | C3 |
|---|---|---|---|---|---|---|---|---|
| Grass | 21.9 ± 5.6 | 21.1 ± 2.5 | 17.8 ± 1.9 | 17.3 ± 2.8 | | NS | NS | NS |
| Leucaena | - | - | 5.0 ±1.3 | 4.2 ± 1.3 | | NS | NS | NS |
| Maiz | - | - | - | 2.0 ± 0.17 | | na | Na | Na |
| Total DMI | 21.9 ±5.69 | 21.1 ± 2.5 | 22.8 ± 2.2 | 23.2 ± 3.1 | | NS | NS | NS |
| Milk Yield | 7.3 | 7.5 | 8.0 | 8.5 | 0.70 | NS | NS | NS |
| Lactose | 44.8 | 44.9 | 53.2 | 53.7 | 1.80 | NS | * | NS |
| Fat | 36.6 | 32.5 | 41.3 | 40.8 | 4.00 | NS | * | NS |
| Protein | 34.2 | 33.4 | 34.0 | 34.2 | 1.60 | NS | NS | NS |
| Urea | 29.4 | 32.5 | 35.3 | 32.5 | 2.28 | NS | NS | NS |

Grass= grass only, SP= silvopastoral system
SP-L= SP without access to Leucaena
SP+M= SP with maize grain supplementation
C1= grass vs SP-L, C2= SP-L vs SP, C3= SP vs SP+M

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# Effects of phytogenics and organic acids alone and in combination on growth performance of weaned piglets

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**Introduction** Intensive research has been directed to the potential of Natural Growth Promoters (NGPs) to replace antibiotics. Phytogenics and organic acids (OA) have been shown to be effective in reducing the incidence of gastrointestinal disorders, thereby improving growth performance in pigs (Steiner, 2006). The addition of OA to nonruminant diets is supposed to have beneficial effects on feed safety since OA have a detrimental impact on moulds and other feed contaminants. Moreover, due to a decrease in gastric pH, acidification of the diets creates favourable conditions for nutrient digestibility, especially in young piglets (Radcliffe *et al.*, 1998). Dietary supplementation with essential oils originating from aromatic plants may directly affect the intestinal microflora, both in quantitative and qualitative terms. As shown under *in vitro* (Helander *et al.*, 1998) and *in vivo* conditions (Kroismayr *et al.*, 2005), oregano essential oils have strong antimicrobial properties. Finally, it has been confirmed that addition of fructooligosaccharides (FOS) to diets for nonruminants may stabilize the gut microflora by selectively supporting the growth of beneficial bacteria (Macfarlane *et al.*, 2006). Depending on individual farm conditions, a well-adjusted combination of different strategies is supposed to maximize the efficacy of NGPs in antibiotic-free feeding systems. The aim of the trial was to investigate the effects of phytogenics and OA alone and in combination in comparison with a commercial diet containing no additives.

**Materials and methods** A scientific trial was carried out at the Centre for Applied Animal Nutrition (CAN), Mank, Austria. 120 weaned piglets with an average initial BW of 6.99 kg were allocated to 12 pens with ten piglets per pen. The animals were randomly assigned to four treatment groups with 30 animals per group. Feed and drinking water were offered *ad libitum*. Body weights of each piglet were recorded at the beginning (day 1), on day 14 and at the end of the trial (day 56). Feed consumption, feeding frequency and mixing ratio were recorded automatically. Treatments were (1) Control (no additives), (2) phytogenics (1 g/kg Biomin® P.E.P. 1000), (3) OA (3 g/kg Biotronic® SE forte) and (4) phytogenics (1 g/kg) + OA (3 g/kg). Biotronic® SE forte is an organic acid blend of formic acid and propionic acid and its salts, based on a sequential release medium. The phytogenic formula used in this study (Biomin® P.E.P.) is based on a blend of fructooligosaccharides and essential oils originating from citrus, oregano and anis. Performance data were subjected to analysis of variance using the statistical software package SPSS 14.0. Pen represented the experimental unit. Treatment effects were determined using the Tukey test.

**Results** As shown in Table 1, supplementation of the basal diet with OA and phytogenics improved average daily gain (ADG) and feed conversion ratio (FCR). From day 1 to day 14, differences in ADG were significant ($P<0.05$), when the phytogenic blend was included in the diets. During the same period, supplementation with OA tended ($P<0.1$) to increase ADG. The highest increase in ADG (4.1%), measured from day 1 to 56, was observed when phytogenics and OA were used in combination. However, this increase was not significant ($P>0.05$).

**Table 1** Effect of phytogenics and OA alone or in combination on performance parameters in piglets

| | Control | Phytogenics | OA | Phytogenics+OA | S.E.M. |
|---|---|---|---|---|---|
| Initial BW day 1 (kg) | 6.97 | 6.99 | 7.00 | 7.01 | 0.109 |
| Final BW day 56 (kg) | 32.84 | 33.25 | 33.38 | 33.95 | 0.422 |
| ADG (g) Day 1–14 | 165[b] | 198[a] | 196[ab] | 178[ab] | 4.4 |
| ADG (g) Day 1–56 | 462 | 469 | 471 | 481 | 6.3 |
| FCR Day 1–14 | 1.53 | 1.07 | 1.30 | 1.24 | 0.118 |
| FCR Day 1–56 | 2.17 | 1.67 | 1.80 | 1.76 | 0.108 |

[a,b] $P< 0.05$

**Conclusions** The use of suitable phytogenics and organic acids may successfully improve growth performance in weaned piglets, thus contributing to the overall productivity in pig production.

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# Effects of an inorganic acid blend on performance and health status in weaned piglets under Vietnamese conditions

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**Introduction** At weaning piglets are exposed to physiological and environmental stress, which often result in reduced feed intake and little or no weight gain (Ravindran and Kornegay, 1993). During the last few decades, diets for weaning piglets have been boosted with various antibiotics in prophylactic doses against gastrointestinal disorders in order to obtain economic benefits due to improved growth rates (4 to 15%) and feed efficiency (2 to 6%; Mroz, 2003). However, growing public concern about the use of antibiotics in animal agriculture and the risk of developing cross-resistance of pathogens to antibiotics used in human therapy has prompted the pig industry to look for alternatives to antibiotic growth promoters that will give similar pig performance. The objective of this study was to evaluate the potential of inorganic acids (phosphoric acid) for nursery pig diets in order to determine their effects on weight gain, diarrhoea and resistance to illness.

**Material and methods** A trial was conducted on a commercial pig farm in Vietnam. An acidifier based on phosphoric acid on a silica carrier (3 kg per t of feed) was tested against a commercial piglet diet containing no acidifying additive. Feed and water were available *ad libitum*. During the trial 60 post-weaning piglets at 26 days of age (hybrid of Yorkshire x Landrace x Duroc) were assigned to two different groups. Pigs were allotted based on same-age, sex and weight. The groups included a basal diet as negative control containing no antibiotics and a treatment with an inorganic acid blend. The number of pigs per pen was 10 with 3 replicates each. The experimental period lasted for 28 days. Data were subjected to biological statistics, using software Excel and Minilab for statistics at significance level of $p < 0.05$. Diarrhoea ratio was determined with the following formula: (total days of diarrhoea / total piglets x trial time (d)) x 100. Results are given as Mean ± SD.

**Results** The results showed that a significant improvement in the acidifier-treatment vs. control in both average weight and weight gain was achieved (Table 1). The final weight in the acidifier-treatment was 15.25 ± 1.99 kg, while the negative control group had a final weight of 13.94 ± 1.91 kg. This body mass difference of more than 1.3 kg (increasing the final weight by nearly 9.4%) in the treatment group after 4 weeks is statistically significant ($p<0.01$). The overall weight gain in the treatment group was also significantly higher ($p<0.01$) compared to the control (7.22 kg v. 5.94 kg).
The difference in feed consumption between the two groups was not statistically significant (10.50 v. 10.60) ($p>0.05$). However, its FCR was significantly ($p<0.01$) lower by nearly 17% (1.47 vs. 1.77) in pigs fed the acidifier.

**Table 1** Performance parameters

| Parameter | Control | Treatment | p-level |
|---|---|---|---|
| Average weight, kg | 13.94 ± 1.91 | 15.25 ± 1.99 | < 0.01 |
| Feed consumed / piglet, kg | 10.50 | 10.60 | > 0.05 |
| Average weight gain, kg | 5.94 ± 0.63 | 7.22 ± 0.71 | < 0.01 |
| FCR | 1.77 | 1.47 | < 0.01 |

Illness and diarrhoea ratios in the treatment group were on average 50% lower than those in the control group, but only for the diarrhoea ratio were these statistically significant ($p<0.05$). No mortality occurred during the trial in either group.

**Conclusion** Acidifiers in animal feed may be used in piglets to compliment their limited capacity to maintain a low gastric pH, and support therefore pepsin activity and digestion. Dietary acidifiers can be accepted as potential alternatives to antibiotics in order to improve performance and health status of livestock. Particularly for post weaning piglets this holds true for the application of acidifiers based on inorganic acids, especially phosphoric acid. It can be concluded that the use of the inorganic acidifier significantly improved performance parameters and health status of treated piglets under Vietnamese conditions.

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# Dietary zinc oxide supplementation in weaner piglets does not cause differential expression of digestive enzymes

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**Introduction** Piglet weaner diets are often supplemented with the antimicrobial mineral zinc oxide (ZnO) to minimise the characteristic growth check post weaning in commercial systems. Zinc has been shown to decrease the incidence of scours, maintain gut morphology and feed intake, and increase growth rate, although the mechanism for these effects remains unclear. Weaning is often accompanied with a decrease in digestive enzyme production by enterocytes, decreasing digestive and absorptive capacity. Digestive enzyme requirements also alter with the dietary change from milk to a solid cereal-based diet, and improvements in enzymatic adaptations may increase digestion and absorption and improve feed utilisation. It is hypothesised in this study that the growth effects of ZnO are linked to improvement in digestion by altering digestive enzyme expression and activity. The aim of this study was to investigate whether the effects of ZnO are linked to expression of the digestive enzymes lactase phlorizin hydrolase (LPH) and aminopeptidase N (ANPEP) in the small intestine (S.I.).

**Material and Methods** Large White x Landrace litters were randomly split in half at weaning and weaned onto a basal diet containing 100 ppm or onto the same diet with supplemented ZnO (3200 ppm). All piglets were weighed weekly, and representative piglets from each treatment were selected for post mortem small intestinal sampling at both 6 and 13 days post-weaning (n=128). Intestinal sections were collected from quartile intestinal lengths, along with corresponding Peyer's patches, and snap frozen. RNA was extracted and reverse transcribed.
Quantitative real-time PCR (qRT-PCR) was used to determine expression of the LPH and ANPEP genes.
Real time data was analysed using ANOVA on SPSS v14.

**Results**. Piglets fed diets supplemented with zinc oxide demonstrated a significantly ($P<0.01$) higher growth rate in the first two weeks post-weaning than those on basal diets (Figure 1), and were significantly heavier at week eight post-weaning ($P<0.01$).
ANPEP expression does not significantly decrease along the intestinal tract, whilst LPH decreases dramatically from the duodenum to the ileum (Table 1a), in accordance with other work (Torp *et al* 1993). However, no effect of treatment was seen on expression of either of the studied genes (Table 1b).

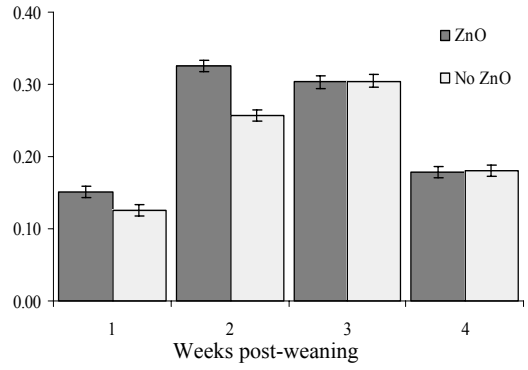


**Figure 1:** Average daily gain post-weaning of piglets fed basal or ZnO supplemented diet

**Table 1a:** Gene expression changes between the proximal and distal small intestine

| | LPH | ANPEP |
|---|---|---|
| Mean Δfold from proximal to distal S.I. | 372 | 1.25 |
| S.E.M. | 56.1 | 0.17 |
| P. value | <0.01 | 0.313 |

**Table 1b:** Gene expression changes between basal and ZnO supplemented fed groups

| | LPH | ANPEP |
|---|---|---|
| Mean Δfold between dietary treatments groups | 1.21 | 0.98 |
| S.E.M. | 0.30 | 0.26 |
| P. value | 0.47 | 0.14 |

**Conclusion** Zinc oxide has a clear impact on growth rates post-weaning. However, expression of the studied digestive enzyme genes in the small intestine is not altered by differing concentrations of dietary zinc oxide, indicating that these genes are not affected by or linked to the growth promoting mechanisms seen. Other genes expressed in the small intestine are now under investigation.

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# Sow agonistic behaviour of two breeds moved from individual stalls to an outdoor park

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**Introduction** Public concern about animal welfare has increased both legislation and consumer pressure, to introduce new technical developments in sow's housing. In recent years, there has been increased interest in less intensive production systems for pigs, including outdoor production. The issue of sow's housing and its relation to welfare has been investigated for sometime now (SVC, 1997; McGlone *et al*., 2004). Concerns over animal welfare combined with European based scientific data have led to the progressive removal of gestation stalls in several European countries, and from 2013 the use of stalls will be restricted throughout the European Union. Therefore one of the greatest challenges in swine housing management is the development of a viable alternative to stalls. In this way, we carried out a study to (1) compare the agonistic behaviour of two breeds of sows moved from individual stalls to an outdoor park, and to (2) determine the evolution of behaviour throughout four weeks of study for each breed.

**Material and methods** The study comprised two experiments, one for each breed, on agonistic behaviour after mixing sows from the stalls to an outdoor park. Each day, over a period of 8 weeks (4 weeks for each breed), 27 multiparous sows of the local breed Bisaro and 22 multiparous sows from a commercial line (Landrace x Large-White) were moved from the individual gestation stalls (2.20 m x 0.60 m) to a 960 $m^2$ outdoor park with grass and rocky ground. Before this study the sows were housed indoor in gestation stalls. The sows were moved to the outdoor park at 09:00 and returned to the gestation stalls at 16:00. From 16:00 to 09:00 the sows were kept in the gestation stalls. Sows were fed twice daily with equal meals (2.0 kg) at 08:30 in the morning and 16:00 in the afternoon. Agonistic behaviour (mutual pushing parallel/inverse or perpendicular position accompanied by bites) observations were done with a video recording camera. The behaviour of each sow was continuously recorded using focal animal sampling (Altmann, 1974).

**Results** Agonistic behaviours were performed more frequently during the first week ($P<0.05$), and became less frequent at the third and fourth weeks for both breeds (Figure 1). Sows from the commercial breed engaged in more agonistic actions ($P<0.05$) than the sows from Bisaro breed (Table 1). Yet, all those aggressive behaviours were mainly observed during the first and second week and after the hierarchy was established the group became stable.

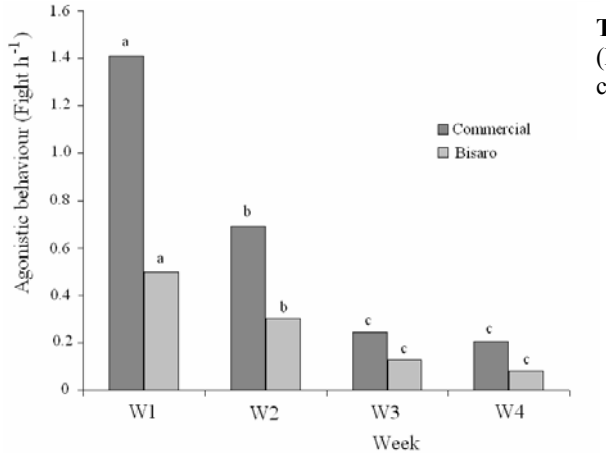


**Figure 1** Agonistic behaviour (Fight $h^{-1}$) for Bisaro and Commercia sows along observation time. Between weeks, bars with differen letters are significantly different at the 5% level.

**Table 1** Least square means for agonistic behaviour (Fight $h^{-1}$). Within an effect, means without a common superscript letter differ ($P < 0.05$).

| Effect | Fight $h^{-1}$ |
|---|---|
| Breed | |
| Commercial | $0.637^{a}$ |
| Bisaro | $0.253^{b}$ |
| Week | |
| W1 | $0.954^{a}$ |
| W2 | $0.497^{b}$ |
| W3 | $0.185^{c}$ |
| W4 | $0.143^{c}$ |
| Probability | |
| Breed | 0.01 |
| Week | 0.01 |

**Conclusions** The outdoor park appeared to stimulate the social and physical interaction between sows, and led to agonistic behaviour, particularly at the first week. Nevertheless, this behaviour shows an effective decrease after the second week, and therefore, allows sows to express most normal patterns of behaviour. The results also, strongly suggest that there are breed differences on agonistic behaviour. This study shows that is possible combine an indoor structure to feed sows and an outdoor park for exercise and social interactions of animals.

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# Reducing the psychological stress of slaughter-weight pigs at loading through targeted enrichment

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**Introduction** The psychological disturbance caused to pigs at loading is thought to be responsible for increased difficulty in handling and impaired well-being through associated rough handling and injury (Geverink *et al*., 1998). Environmental enrichment (toys and driving) has been shown to reduce the force required and the excitability of pigs driven through a chute (Grandin *et al*., 1986). The objective of the current study was to investigate whether it is possible to reduce the psychological stress of slaughter-weight pigs at loading by reducing the novelty of the loading process.

**Materials and methods** Large White x Landrace fattening pigs (n = 112) were used in the study which ran in two periods, each of three weeks in length. Pigs were weighed and selected three weeks off slaughter weight and were split into pen groups containing eight pigs, balanced for sex and weight (79.37kg ± 0.15kg, mean ± s.e.). Treatment **(T)** and control **(C)** animals were housed in separate buildings (changed over for second replicate) and all pens provided with straw. Following a one week rest period T pigs received a two-week treatment period (days 1-14). The total package of enrichment for T pigs was designed to include simple and novel yet progressively challenging stimuli containing relevance to the loading situation. C pigs received no treatments and remained as standard commercially reared pigs. Enrichment was given to T pigs each morning between 0800 h and 0900 h after pen cleaning. A brass bell was rung for 30 seconds to act as a cue for environmental change. Pens then received the enrichment simultaneously in the order indicated in table 1. In addition, T pigs received increased human visual contact as the researcher observed the pigs during the administration of the enrichment.

**Table 1** Environmental enrichment and days given to the T pigs throughout the two week enrichment programme

| Stimulus | Enrichment (days administered) |
|---|---|
| Initial novelty | Chain (1), natural rope (2) and remaining in pen thereafter. Tarpaulin consecutively presented for 20 minutes (3, 4, 5, 12 and 13). |
| Sound | Collection of commercial loading sounds played for 20 minutes at 60dB (6), 70dB (7), and 75-80dB (8, 10, 11, 12 and 13). |
| Loading ramp | Hinged, checker plate ramp at angle of 8° installed in pens (9) remaining in pen. |
| Handling | One movement out of pens (9) when ramps were installed. |

At 19 days following selection both T and C pigs were re-weighed. The response of pigs to loading was tested by loading each T and C pen group onto a trailer. The time taken for the 1st pig, 50% of the pen group and the total pen group to load was recorded. The number of refusals seen per pen group was also noted. Pigs remained on the trailer for 10 minutes and were then returned to pens. A defecation score was taken from the inside of the trailer by counting the intact piles of faeces. The trailer was cleaned between groups. For analysis, data was checked for normality and non-normal data $\log_{10}$ transformed. Results from one pen group of treatment pigs were omitted due to an error at loading. All data was analysed with a one-way analysis of variance with treatment as a factor.

**Results** In the times taken to load onto the trailer T groups showed a tendency to be quicker for the first and 50% of the pigs to load onto the trailer (Table 2).

**Table 2** Time taken (s) by T (n = 6 pen groups) and C (n = 7 pen groups) groups to load (mean and pooled s.e.)

| Loading (s) | Treatment | Control | S.E. | *P* |
|---|---|---|---|---|
| 1st pig | 4.33 | 8.14 | 0.11 | 0.09 |
| 50% of pen group | 6.67 | 12.29 | 2.13 | 0.08 |
| Total pen group | 9.67 | 15.14 | 2.49 | 0.13 |

There were no significant differences between the T and C groups in the refusals seen on the ramp at loading ($P>0.05$). However, pigs that had received the enrichment had a significantly lower defecation score in response to loading (T: 7.71 ± 1.11, C: 12.71 ± 1.70, mean ± s.e., $P<0.05$). At the end of each study period the average pen weight gain between the T and C pigs showed a tendency for pens of T pigs to have a greater increase in weight gain (T: 15.72kg ± 0.51kg, C: 13.90kg ± 0.82kg, mean ± s.e., $P = 0.08$).

**Conclusion** The reduced defecation score of the T pigs suggests that the enrichment was effective at reducing the emotional reactivity of the pigs towards loading. Although no significant differences were observed in refusals displayed, a reduction in fear could account for the tendencies of T pigs to be quicker to load. Further work is required to elucidate whether the observed weight gains in the T group are of serious economic significance.

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## Sustainable utilisation of cassava plant for feeding monogastric animals

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**Introduction** Nigeria, which produces an estimated 34 million metric tons of cassava annually, is the leading producer of cassava world-wide (FAO, 2004a). There have been several studies by many scientists on the use of cassava for livestock feeding. Most of these studies centred on the use of either flour or peels or leaves. Besides, most of these studies confirmed the suitability of cassava flour to replace maize partially or wholly in the diets of all species of livestock. The replacement of maize with cassava flour was reported to be economical. These findings appeared to have been over taken by events and recently in Nigeria, cassava has been attracting interest as an industrial crop having found various uses in the starch, pharmaceutical, bread, and biscuit industries. This has made the price of cassava flour to be on the increase. Based on the high cost of cassava flour, its use to replace maize is becoming unattractive economically. As a result of this, attempt was made to reduce the flour content in the diets of monogastric animals considerably by adding more of peels, leaves, and tender-stems. Most of these by products from cassava especially peel, leaves, and tender-stems are under-utilised as they are often left to rot away after harvest on farms and homesteads where cassava is grown in Nigeria. Hence, the objective of these studies was to evaluate the effect of inclusion of various products and by products obtainable from cassava in a single or composite diet on the performance of monogastric animals.

**Materials and methods** Cassava plant meal (CPM) which had about 9.0% crude protein (CP) comprising unpeeled tubers, leaves and tender-stems was developed. The mixing ratios were 2.5:1 of unpeeled tubers to leaves plus tender- stems, while the ratio of leaves to tender-stems was 5:1.CPM was used as maize replacer in the diets of rabbits, pigs, cockerels and broilers. Growth studies were conducted which lasted for eight weeks with rabbits, eight weeks with pigs, sixteen weeks cockerels and four weeks with broilers. In the broiler study, a basal diet of 22%CP was formulated with 50% maize. The maize was replaced with CPM at rates 0, 25 and 50 % of maize. A total of 153, one week old broiler chicks were randomly allotted to 3 experimental diets with each diet having 3 replicates. In the study with rabbits, 15 ten-week old new Zealand white weaner rabbits were randomly allotted to 3 diets formulated to contain about 15% CP with CPM replacing 0, 50 and 100 % of maize in the diets.5 animals were used per treatment with each serving as a replicate. In the study with cockerels, 3 diets that contained about 16% CP and 10.37MJ metabolisable energy were formulated with CPM replacing 0, 50 and 100% of maize in the diets. 150 day old cockerel chicks were randomly allotted in the 3 diets with each diet having 3 replicates. 24 growing pigs were used in the pig study and they were randomly distributed into 3 diets that contained about 18% CP and 11.2 MJ digestible energy.6 animals were used per treatment with each serving as a replicate in the pig study. CPM was used at 0, 50 and 100% of maize. The design of the studies was completely randomised design. Proximate compositions of CPM and experimental diets were carried out according to AOAC (1995), while the residual cyanide content of CPM was determined as outlined by ISO (1975). Routine management practices were followed. Records of feed consumption, weight gain and feed/gain ratio were kept on treatment basis. All data were subjected to analysis of variance using a computer software package (SAS2000). There was no lesion or physical disability arising from the treatment effect.

**Results** The diets fed each species had a similar proximate analysis with only slightly higher levels of crude fibre as the proportion of CPM was increased. The residual cyanide content of CPM was 33.5mg/kg.The performance data are shown on the Tables below. The feed intake was calculated on dry matter (DM) basis

**Table 1***Effect of CPM in the diets of broiler chicks*

| | 0 CPM | 12.5CPM | 25CPM | SEM |
|---|---|---|---|---|
| Daily gain,g/day | $36.0^{a}$ | $31.4^{b}$ | $29.1^{b}$ | 2.9 |
| DM intake,g/day | $452^{a}$ | $448^{a}$ | $460^{a}$ | 4.8 |
| Feed/gain ratio | $1.79^{a}$ | $2.04^{b}$ | $2.26^{b}$ | 0.19 |

**Table 2** *Effect of CPM in the diets of pigs*

| | 0CPM | 25CPM | 50CPM | SEM |
|---|---|---|---|---|
| Daily gain,g/day | $360^{a}$ | $300^{a}$ | $340^{a}$ | 17.64 |
| DM intake,g/day | $890^{a}$ | $940^{a}$ | $960^{a}$ | 20.82 |
| Feed/gain ratio | $2.5^{a}$ | $3.00^{a}$ | $2.78^{a}$ | 0.15 |

**Table 3** *Effect of CPM in the diets of cockerels*

| | 0 CPM | 25 CPM | 50 CPM | SEM |
|---|---|---|---|---|
| Daily gain,g/day | $19.28^{a}$ | $16.05^{b}$ | $12.88^{c}$ | 2.61 |
| DM intake,g/day | $99.21^{a}$ | $99.60^{a}$ | $100.01^{a}$ | 0.50 |
| Feed/gain ratio | $5.15^{c}$ | $6.21^{b}$ | $7.77^{a}$ | 0.12 |

**Table 4** *Effect of CPM in the diets of rabbits*

| | 0CPM | 22.5CPM | 45CPM | SEM |
|---|---|---|---|---|
| Daily gain,g/day | $9.38^{b}$ | $12.40^{a}$ | $11.20^{ab}$ | 1.55 |
| DM intake,g/day | $43.90^{b}$ | $56.4^{a}$ | $60.80^{a}$ | 8.81 |
| Feed/gain ratio | $5.15^{a}$ | $4.89^{a}$ | $6.00^{a}$ | 0.47 |

For all the Tables, means along the same row having different superscripts differ at $p<0.05$.
In the study with broilers, the growth rate decreased and feed to gain ratio deteriorated as the proportion of the CPM in the diet was increased. In the study with pigs, the daily gain, feed intake and feed/gain ratio were not significantly ($p>0.05$) influenced by the inclusion of CPM to replace maize in their diets. In the study with cockerels, daily gain and feed/gain ratio were significantly ($p<0.05$) affected when CPM replaced maize in their diets. The inclusion of CPM to replace maize in the diets of growing rabbits resulted in improved performance of the animals in terms of daily gain, feed intake and feed/gain ratio.

**Conclusion** Findings from the above studies suggest the suitability of CPM to replace maize completely especially in the diets of pigs and rabbits. Partial replacement of maize with CPM gave a satisfactory performance with broilers and cockerels. The poorer utilisation of CPM by broilers and cockerels may be due to its high fibre content compared to maize.

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# The effect of a dietary urine acidifier as a solution to improve / prevent infertility problems in sows on a commercial farm in Cyprus

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**Introduction** Reproductive problems in sows are the cause for at least 15% of the sow culls in Cyprus. The main cause of reproductive failure is urogenital tract infections (UTI), caused by pathogenic bacteria entering the sow's reproductive tract, especially at risk times of farrowing and serving when the cervix is open (Almond and Richards 1992). Indigenous people have used cranberry preparations to prevent UTIs and other illness for centuries (http://www.cranberryinstitute.org/health/urinarytract.htm). An endogenous mechanism of the sow as protection against bacterial infections in the urogenital tract is the urinary pH, which can be manipulated by dietary means affecting the dietary anion-cation balance (Beker 1999). The objective of this experiment was to evaluate the effect of a dietary urine acidifier based on acidic acting substances (mixture of phosphoric acid and selected ingredients containing anions) and cranberry extract on sow urine parameters (pH, leucocytes and nitrates) and sow fertility parameters.

**Materials and Methods** A total of 30 Landrace X Large White pregnant breeding sows of similar parity were split into two groups on a commercial farm in Cyprus. The control group was fed conventionally throughout pregnancy and lactation. The trial group was fed conventionally until day 107 of pregnancy. However from day 108 of pregnancy until day 7 of lactation and from day 3 pre-weaning until A.I. service the trial group received a dietary urine acidifier based on anionic substances and cranberry extract (Biomin® pHD) as a top dressing at 20g/sow/day to the conventional diet. Lactation length (31 days), environment, and management practises were standardised for both groups. Measurements included urinary parameters such as pH at day 108 of pregnancy, 1 day post farrowing, 7 days post farrowing, 5 days pre weaning and 1 day post weaning, as well as urinary nitrites and leucocytes. Urinary pH was measured using a pocket pH meter (ad II0 pH) from samples that were collected without contamination. Urine nitrites and leucocytes were measured with test strips for urine analysis (Conbur Test® stripes Roche). Data were analysed by one-way analysis of variance using dietary treatment as a factor (Minitab, version 14). Urinary pH prior to treatment was used as a covariate in analysis of urinary pH changes.

**Results** At the beginning of the trial the overall mean for the urinary pH in sows was 6.6 (±0.0716 SEM) and similar for both groups (Table 1). Urinary pH decreased between day 108 of pregnancy and farrowing only in the trial group, giving a significant difference in pH at farrowing ($P<0.05$). In this limited sample, the correlation between the number of days of Biomin® pHD in the sow's diet pre-farrowing (range 4-10 days) and the change in urinary pH at farrowing was not significant ($r=0.52$, $P=0.12$). Urinary pH continued to decrease for the trial group in the 7 days post farrowing, in contrast to the control group, which showed an increase. As a result there was a significant difference between the two groups at 7 days post farrowing ($P<0.001$). After the supplement was withdrawn, the difference disappeared, so that groups were similar by 5 days pre-weaning. After reinstatement of the supplement for 4 days, there was again a significant difference ($P=0.001$) between the two groups by 1 day post weaning. No differences between groups were seen in the prevalence of clinical post farrowing health disorders or urinary indicators of reproductive tract infection, which were low in both groups.

**Table 1** Effects of a dietary urine acidifier on urinary pH

| | Control | Biomin® pHD | P-value | SED |
|---|---|---|---|---|
| No. of sows | 15 | 15 | - | - |
| Parity | 3.5 | 3.2 | 0.705 | 0.70 |
| pH on d108 pregnancy (1) | 6.7 | 6.6 | 0.585 | 0.145 |
| pH at farrowing (2) | 6.7 | 6.3 | 0.031 | 0.192 |
| pH at d7 post farrowing (3) | 7.2 | 6.1 | <0.001 | 0.173 |
| pH at 5 days pre weaning (4) | 6.5 | 6.6 | 0.723 | 0.330 |
| pH at 1 day post weaning (5) | 6.7 | 5.7 | 0.001 | 0.231 |
| pH (2) – pH (1) | 0.15 | -0.23 | 0.119 | 0.230 |
| pH (3) – pH (1) | 0.59 | -0.46 | <0.001 | 0.230 |
| pH (5) – pH (4) | 0.23 | -0.63 | 0.022 | 0.328 |

**Conclusion** The addition of a dietary urine acidifier reliably reduced urine pH at critical times in the reproductive cycle. The consequences of this for subsequent fertility (weaning to conception interval and litter size) are currently being evaluated.

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# Study for the validation of the n-alkane technique to estimate intake and digestibility of acorns and pasture by "Alentejano pigs"

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**Introduction** The sustainable animal production systems, based on pastures require the knowledge of animal and vegetation responses to different management strategies (Oliván and Osoro, 1997). The lack of knowledge about the grazing behaviour, intake and diet digestibility of "Alentejano pigs" explored under "Montanheira", leads to many doubts about the most adequate production strategies. N-alkanes are components of plant cuticular wax and have been successfully used as markers for estimation of intake in ruminants (Dove and Mayes, 1991), however few studies with pigs have been published, particularly "alentejano pigs". In the "Montanheira" system, the main components of the diet are acorns and pasture. Therefore this trial was conducted to validate the use of the n-alkane technique to estimate intake and digestibility of the particular diet composition of "Montanheira" (acorns and pasture) by "Alentejano pigs".

**Material and methods** Eight male "Alentejano" pigs (average LW 58 kg) were housed in metabolic cages, in an environmentally controlled room. The animals were randomly allocated in two groups. Group 1 received one 20g cake (maize flour and sugar cane syrup) per animal per day containing 100 mg of $C_{32}$ and 150 mg of $C_{36}$ and group 2 received two cakes per animal per day containing 50 mg of $C_{32}$ and 75 mg of $C_{36}$. The animals were fed, with 400g of grass and 3kg of acorns per day (fresh weight), distributed in two meals (09.30 and 17.30h). Faecal samples were collected daily, for ten days, from the first day of n-alkanes administration, to determine the pattern of excretion of the markers. Total faecal collection was carried out during the last 5 days of the trial, to determine faecal n-alkane recoveries. Within this period, faecal samples were collected every four hours, per *rectum* for 3 days to measure the daily excretion pattern of n-alkanes. Intake and digestibility were measured *in vivo*, per animal, during 5 days and estimated using the n-alkane technique. Data collected were submitted to factorial ANOVA (STATISTICA™ 6.0, Statsoft®). When one factor or the interaction of two factors were not significant, one way ANOVA was carried out. Treatment means were compared using LSD (Least Significant Difference).

**Results** One animal was withdrawn from the trial due to illness during the adaptation period. The frequency of administration of artificial alkanes (once or twice daily) did not significantly ($P>0.05$) affect the patterns of excretion of artificial n-alkanes nor estimates of intake and digestibility, therefore, a single group with eight animals was considered in this experiment. The refusal of grass was high comparing to that of acorn. The faecal excretion of the artificial n-alkanes stabilized on the fifth day after the first dosing of alkanes. No daily variation was observed in the faecal concentrations of even-chain and odd chain n-alkanes. Concentrations of n-alkanes in acorn and grass and mean faecal n-alkane recoveries are shown in Table 1. Given the high mean standard error (10.7) obtained, the recoveries of $C_{27}$, $C_{29}$ and $C_{31}$ were not significantly ($P>0.05$) different from at least one of the artificial n-alkanes ($C_{32}$ or $C_{36}$).

**Table 1** N-alkanes concentration in acorn and grass and faecal recovery of n-alkanes (n=7)

| n-alkane | $C_{25}$ | $C_{27}$ | $C_{29}$ | $C_{31}$ | $C_{32}$ | $C_{33}$ | $C_{36}$ | SEM |
|---|---|---|---|---|---|---|---|---|
| Acorns (mg/kg DM) | 9.5 | 31.9 | 47.1 | 4.4 | 2.0 | 1.0 | 1.5 | |
| Grass (mg/kg DM) | 15.5 | 37.8 | 176.7 | 231.1 | 9.3 | 39.6 | 7.6 | |
| Faecal Recovery (%) | 13.1$^{a}$ | 75.1$^{be}$ | 133.9$^{c}$ | 91.5$^{bd}$ | 106.8$^{bc}$ | 56.8$^{e}$ | 109.9$^{cd}$ | 10.7 |

$^{a-e}$ Means within the same row with no common superscript are significantly different ($P<0.05$)

The determination and estimates of intake and digestibility are shown in Table 2. The pairs $C_{29}$:$C_{32}$ and $C_{29}$:$C_{36}$ gave the best estimates of the measured intake and digestibility.

**Table 2** Digestibility *in vivo* and estimated from intake (using an artificial and a natural n-alkane) and faecal production (using an external alkane as a marker) and intake *in vivo* and estimated pairs of artificial/natural alkanes.

| | Estimates | | | | | Measured | n | SEM |
|---|---|---|---|---|---|---|---|---|
| | $C_{32}$:$C_{27}$ | $C_{32}$:$C_{29}$ | $C_{32}$:$C_{31}$ | $C_{36}$:$C_{29}$ | $C_{36}$:$C_{31}$ | *in vivo* | | |
| Digestibility | 0.849$^{ac}$ | 0.916$^{b}$ | 0.852$^{ac}$ | 0.909$^{ab}$ | 0.844$^{c}$ | 0.892$^{abc}$ | 7 | 0.0342 |
| Intake (kg DM) | 3.75$^{a}$ | 6.37$^{b}$ | 4.95$^{ab}$ | 6.22$^{b}$ | 4.92$^{ab}$ | 6.35$^{b}$ | 7 | 0.499 |

$^{a-c}$ Means within the same row with no common superscript are significantly different ($P<0.05$)

**Conclusions** No daily variation was observed in the faecal concentrations of even-chain and odd chain n-alkanes, therefore indicating that, in similar experimental conditions, intake and digestibility estimates can be based upon only one daily faecal sample collection. Estimates of intake and digestibility were not influenced by the frequency of administration of artificial n-alkanes. It is possible to estimate intake and digestibility of acorn and grass in extensive conditions with the n-alkane technique. Low concentration of n-alkanes in acorns, and the low intake of grass observed during the experiment were probably responsible for the high standard errors observed in our results.

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# Evaluation of artichoke (*Cynara scolymus*) by-product using the semi- automatic *In vitro* gas production technique

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**Introduction** The major constraint limiting the development of livestock production in many developing countries is inadequacy of animal feed; although there are a wide range of valuable by-products and residues eligible to complement it. To estimate whether a material is appropriate for animal feeding, *in vitro* gas production (GP) technique have been used to evaluate the potential of different classes of feedstuffs to supply nutrients to ruminants. Thus, the fermentation characteristics of artichoke by-product (*Cynara scolymus*), alfalfa and Tifton 85 (*Cynodon sp*) hay were evaluated by *in vitro* GP technique.

**Materials and methods** Artichoke (*Cynara scolymus* L.) by product, alfalfa hay and Tifton-85 (*Cynodon sp*) hay (TIF) were obtained from local dealers. Feedstuffs were analyzed according to AOAC (1995) and Van Soest et al., (1991), as well analyzed for extractable total phenols (TP), tannins (TT) and condensed tannins (CT) according to Makkar et al., (1993). The *in vitro* GP assay was carried out using a pressure transducer, 160 mL serum bottles incubated at 3, 6, 9, 14, 24, 36, 48, 60, 72 and 96 h at 39ºC and inoculum from five sheep as for Bueno et al., (2005). About 0.5 g of each sample was incubated in buffered rumen fluid (2:1, v/v). Three GP runs were done with four replicates for each sample. Dry and organic matter degradability were measured at 24 h. GP from soluble and non-soluble fraction were calculated according to Van Gelder et al., (2005). Efficiency of microbial protein *in vitro* was estimated by the mg of truly organic matter degradation: gas volume produced thereby (termed as a partitioning factor, PF (mg/ml) at 24 h incubation) according to Blummel et al., (1997). Means were compared by Tukey test.

**Results** The crude protein contents were 93.8, 150.1 and 181.9 g $kg^{-1}$ DM for TIF, artichoke by-product and alfalfa, respectively. The NDF was significantly higher in TIF than artichoke by product and alfalfa. The secondary compounds analyses resulted in negligible contents in all tested feeds. There were significant differences among feeds in asymptotic GP. The highest cumulative GP was observed in artichoke by-product which was followed by TIF and alfalfa. Artichoke by-product produced gas by 20 % from soluble fractions and 80 % from insoluble fractions; while alfalfa and TIF were produced gas by 9 and 6 % from soluble fractions and 91 and 94 % from insoluble fractions respectively. There were significant ($P < 0.01$) differences among investigated roughages in the dry and organic matter degradation *in vitro*, with artichoke-by product showing the highest values. PF values did not significantly differ ($P>0.05$) between artichoke by-product and alfalfa (3.6 and 3.2 mg true digested organic matter/ml gas) but it was low in TIF (2.7 mg true digested organic matter/ml gas).

**Table 1** The chemical composiion (g/kg DM) of experimental materials**.**

| | CP | NDF | ADF | TP | TT | CT |
|---|---|---|---|---|---|---|
| Artichoke | 150 | 524 | 412 | 12.7 | 8.1 | 0.30 |
| Alfalfa | 182 | 547 | 346 | 10.2 | 6.6 | 0.20 |
| Tifton | 94 | 798 | 367 | 7.1 | 4.0 | 0.90 |

Crude protein (CP), neutral-detergent fibre (NDF), acid-detergent fibre (ADF), total phenols (TP), total tannins (TT), condensed tannins (CT).

**Table 2** Potential gas production, estimated kinetic parameters, gas production from soluble (GPSF%) and insoluble fractions (GPNSF%), partition factor (mg true digested organic matter/ml gas), dry and organic matter degradation (g/kg DM) for Artichoke, Alfalfa and Tifton incubated with rumen fluid *in vitro*

| | A, ml/g DM | T (h) | μ24 | μ96 | GPSF | GPNSF | PF | DMD | OMD |
|---|---|---|---|---|---|---|---|---|---|
| Artichoke | $317^{a}$± 4.7 | $1.4^{a}$±0.18 | $0.0290^{a}$ | $0.0145^{a}$ | $20^{a}$±1.4 | $80^{b}$±1.4 | $3.2^{ab}$±0.4 | $716^{a}$±58 | $706^{a}$±60 |
| Alfalfa | $228^{b}$±4.3 | $0.8^{a}$±0.22 | $0.0136^{b}$ | $0.0068^{b}$ | $09^{b}$±5.9 | $91^{a}$±5.9 | $3.6^{a}$±0.3 | $446^{b}$±62 | $415^{b}$±56 |
| Tifton | $275^{b}$±4.8 | $1.5^{a}$±0.09 | $0.0095^{b}$ | $0.0048^{b}$ | $06^{b}$±6.6 | $94^{ab}$±6.9 | $2.7^{b}$±0.5 | $447^{b}$±64 | $386^{b}$±59 |

A: potential gas production; T: lag time; μ: rate of gas production ($h^{-1}$)

**Conclusions** This study suggested that artichoke have potential fermentation efficiency better than alfalfa hay and therefore, artichoke could be incorporated in feed mixtures to replace conventional roughage sources (hay, silage) in ruminant diets without major problem.

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# The effect of mixed enzyme and chemical treatment of bagasse, untreated and steam pre-treated sugarcane pith on *in vitro* digestion of dry matter

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**Introduction** Crop residues are a major source of low-quality biomass that can be fed to ruminants. Sugarcane bagasse and sugarcane pith, the residue after rind removal, are highly lignified by products of the sugar and paper industries, respectively (Muller, 1978). The low digestibility, high lignin and very low nitrogen content, are considered the main reasons for unsatisfactory performance of animals fed this roughage. Steam-pressure treatment cleavage the bounds between lignin and the other component of the cell wall, These are associated with the improved utilization of cell wall polysaccharides in the steam pre-treated materials by cell-free enzymes and enzymes of the rumen microbial ecosystem. The possibility of increasing nutritive value of fibrous feedstuffs by enzymes treatment has been studied in recent years. A number of studies in the 1960s involving cattle and sheep showed that enzymes substantially improved feed digestibility and animal performance, but results were often inconsistent The objectives of our study were to characterize the response of sugarcane bagasse, pith, untreated and steam treated, to sodium hydroxide and enzyme mixtures treatment.

**Materials and methods** The steam treated (STP) sugar cane pith, was prepared at 20 bar for 3 min. Each of roughages was divided into two batches to form duplicate sources of each substrate for treatment with sodium hydroxide (NaOH). Substrates were treated with 4% NaOH. Treatment consisted of suspending 100 g of substrate and 4 g of NaOH in 100ml of distilled water The mixture was at room temperature for 48 h. The enzyme mixture (Bioproton Pty. Ltd. Co.) contained phytase, beta glucanase, alpha amylase, cellulase, hemicellulase, pectinase, xylanase, was added in dry form to untreated (UTP), steam treated (STP) and sodium hydroxide treated (SH) sugarcane pith, 1g/kg DM. Enzyme activities were cellulose at 8200, xylanase at 2000, α-amylase at 3400 IU/g; and pectinase at 900 units of polygalacturonase per gram. Forty-eight-hour *in vitro* Dry matter digestibility (IVDMD or D) of untreated, steam treated pith with and without enzyme and sodium hydroxide treat was determined by Tilly and Terry (1963) method. Statistical analysis of data was done using the SAS (2002) procedures.

**Results** *In vitro* dry matter digestibility of untreated and steam treated sugarcane pith are shown in Table 1. The main effects of steam, sodium hydroxide and enzyme are summarized in Table 2. Treatments with high pressure-steam, rind removal (bagasse *vs*. UTP) of sugarcane bagasse and sodium hydroxide significantly ($P<0.05$) increased digestibility of pith. Regardless to other treatment enzyme numerically caused to increased digestibility ($P>0.05$). A number of studies recently showed that enzymes improved cattle performance, but no improvement was observed in others. Apparently, the inconsistent results from those studies can be contributed to a number of factors, including diet composition, type of enzyme preparation, complement of enzyme activities, and amount of enzyme provided, enzyme stability, and method of application (Yang et. al., 1999). There was a significant interaction between bagasse digestibility and adding of enzyme. Sodium hydroxide processed STP and UTP has the highest DMD and untreated bagasse lowest ($P<0.05$). Treatment with Alkaline Hydrogen Peroxide increased ($P<0.05$) IVDMD, extent of neutral detergent fibre digestion, and monosaccharide digestibility of all crop residues (Amjed, 1992).

**Table 1** Effect of enzyme and sodium hydroxide on IVDMD of untreated and steam treated sugarcane pith

| | Treatment† | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | STPN | UTPNE | FPNE | BN | UTPN | BNE | UTPE | STP | STPE | UTP | BE | B | SEM |
| D | $46^{a}$ | $45^{a}$ | $42^{ab}$ | $41^{ab}$ | $40^{ab}$ | $40^{ab}$ | $34^{bc}$ | $32^{bcd}$ | $30^{cd}$ | $26^{cde}$ | $24^{de}$ | $20^{e}$ | 1.00 |

† Values with different superscripts within columns differ at $P<0.05$ N= NaOH E= Enzyme B= Bagasse

**Table 2** The main effects of steam, sodium hydroxide and enzyme on DMD of sugarcane bagasse and pith†

| | steam | | | NaOH | | enzyme | |
|---|---|---|---|---|---|---|---|
| | bagasse | STP | UTP | 0 | 4% | 0 | 1 |
| IVDMD | $27.33^{b}$ | $31.5^{a}$ | $34.44^{a}$ | $27.73^{a}$ | $42.33^{b}$ | 30.66 | 33.75 |
| SEM | 1.4 | | | 0.98 | | 1.00 | |

† Values with different superscripts within columns differ at $P<0.05$

**Conclusions** The results of the present study demonstrate that treatments with high pressure-steam, rind removal of sugarcane bagasse and use of sodium hydroxide, significantly ($P<0.05$) increased digestibility of sugarcane by-products. Effects of steam treatment have been depend on the different conditions such as pressure, time and moisture. Therefore it is possible that other condition modify DMD (Liu, 2000). Enzyme numerically caused to increased digestibility ($P>0.05$). Using the other enzyme mixture, methods of application and amount of enzyme provided, maybe improved the effects of enzyme on digestibility (Yang et. al., 1999).

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# The relationship between *in vitro* and *in situ* dry matter disappearance of some Iranian feedstuffs in sheep

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**Introduction** Ration formulation systems require information on nutrient requirements of the animal and reliable values for rumen degradable and undegradable fractions of feed ingredients. The *in situ* nylon-bag technique is widely used to characterize the disappearance of feeds from the rumen (Woods et al., 2002). The objective of this study was determining of relationship between *in vitro* and *in situ* dry matter disappearance.

**Materials and methods** Test feeds included corn grain (CG), soybean meal (SBM), alfalfa (AA) and wheat bran (WB). The chemical composition of the test feeds are shown in Table 3. Two ruminally fistulated sheep (38±1.5 kg) used. The sheep were fed a diet consisting of 600 g $kg^{-1}$ concentrate and 400 g $kg^{-1}$ forage containing DE (14.01 MJ $kg^{-1}$ DM) and CP (160 g $kg^{-1}$ DM). Nylon bags which were approximately (6*12 cm) containing 5 g (2mm screen) were incubated in duplicate in the rumen of fistulated sheep for 0, 2, 4, 8, 12, 24, 48h (for CG and SBM) and 72 and 96 h (for AA and WB). Disappearance of test feeds at each incubation time was expressed relative to original feed. Also two ruminally fistulated sheep used as donors of ruminal fluid for preparation of inoculum for *In vitro* experiment. Approximately 300 mg of feed was weighted and placed into a serum bottle. Buffered rumen fluid with McDougal buffer (20 ml) was pipetted into each serum bottle. All the serum bottle incubated for 2, 12, 24 and 48h (3 bottle for each time). *In vitro* disappearance of DM was measured relative to original feed. The rate and extent of DM degradation was estimate according to the equation: $p = a + b(1 - e^{-ct})$. Effective degradability (ED) was calculated according to equation: $ED = a + (b \times c)/(c + k)$, assuming an outflow rate ($k$) of 0.02 $h^{-1}$. The data was analyzed using the ANOVA procedure of SAS (SAS Institute, 1987).

**Results** The results are shown in Tables 1, 2 and 3. There were the differences between chemical composition in test feeds (Table 3). There were significant differences between feedstuffs in incubation times ($P$<0.05). Dry matter disappearance in corn grain at 48h was more than the other feedstuffs. This can be resulted due to low ADF. The DM *in vitro* disappearance in incubation times had significant differences ($P$<0.05). The disappearance of potential fraction (b) in both of *in situ* and *in vitro* methods for CG was more than the other test feeds. These significant differences for DM *in vitro* and *in situ* can be predicted due to different chemical composition of feedstuffs (Table 3). The relationship between *in situ* and *in vitro* for CG ($R^2$ = 0.8692, y = 0.8358x + 3.7146), SBM ($R^2$ = 0.9567, y = 0.9421x + 2.5563), WB ($R^2$ = 0.979, y = 1.0484x - 4.0489) and AA ($R^2$ = 0.9473, y = 1.0724x - 2.4055).

**Table 1** *In situ* mean disappearance of dry matter (%) *

| | Time of incubation (h) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Feeds | 0 | 2 | 4 | 8 | 12 | 24 | 36 | 48 | 72 | 96 | a | b | c(%/h) | ED |
| CG | 16.7$^{b}$ | 18.6$^{d}$ | 28.9$^{c}$ | 40.9$^{b}$ | 51.0$^{b}$ | 64.1$^{a}$ | 67.2$^{a}$ | 73.6$^{a}$ | - | - | 14.0 | 59.8 | 0.07 | 61.1 |
| SBM | 25.8$^{a}$ | 33.9$^{a}$ | 37.2$^{b}$ | 48.5$^{a}$ | 58.5$^{a}$ | 62.0$^{b}$ | 65.3$^{a}$ | 69.0$^{b}$ | - | - | 25.3 | 42.2 | 0.10 | 60.7 |
| WB | 27.2$^{a}$ | 29.6$^{b}$ | 40.2$^{a}$ | 48.2$^{a}$ | 52.0$^{b}$ | 55.3$^{c}$ | 58.3$^{b}$ | 65.5$^{c}$ | 69.2$^{a}$ | 76.5$^{a}$ | 30.3 | 41.9 | 0.04 | 59.1 |
| AA | 17.3$^{b}$ | 20.6$^{c}$ | 24.0$^{d}$ | 28.3$^{c}$ | 31.9$^{c}$ | 40.2$^{d}$ | 45.2$^{c}$ | 50.4$^{d}$ | 55.4$^{b}$ | 57.0$^{b}$ | 18.2 | 40.4 | 0.03 | 43.4 |
| SEM | 0.47 | 0.57 | 0.70 | 0.62 | 0.53 | 0.66 | 0.62 | 0.63 | 0.45 | 0.56 | | | | |

* The means within a column without common letter differ (P<0.05).

**Table 2** *In vitro* mean disappearance of dry matter(%)*

| | Time of incubation (h) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Feeds | 2 | 12 | 24 | 48 | a | b | c(%/h) | ED |
| CG | 18.4$^{b}$ | 56.3$^{a}$ | 66.5$^{a}$ | 69.8$^{a}$ | 13.0 | 58.2 | 1.10 | 61.7 |
| SBM | 37.8$^{a}$ | 50.3$^{b}$ | 60.4$^{b}$ | 71.3$^{a}$ | 29.1 | 44.8 | 0.05 | 61.9 |
| WB | 35.5$^{a}$ | 54.5$^{a}$ | 55.3$^{c}$ | 65.8$^{b}$ | 28.3 | 35.3 | 0.09 | 57.5 |
| AA | 17.8$^{b}$ | 29.1$^{c}$ | 46.3$^{d}$ | 48.8$^{c}$ | 15.5 | 38.5 | 0.04 | 42.8 |
| SEM | 0.78 | 0.69 | 0.61 | 0.61 | - | - | - | - |

**Table 3** Test feeds composition.

| | Items (%DM) | | |
|---|---|---|---|
| feeds | CP | NDF | ADF |
| CG | 11.86 | 49.15 | 6.25 |
| SBM | 49.29 | 44.95 | 13 |
| WB | 16.92 | 53.3 | 15.8 |
| AA | 17 | 49.95 | 40.35 |

* The means within a column without common letter differ (P<0.05).

**Conclusions** There is the high correlation between *in vitro* and *in situ* dry matter disappearance of test feeds resulting that *in vitro* technique can be substituted *by in situ* method.

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# The comparison between nylon bag and gas production method, use of regression equations in determination of feedstuffs nutritive value

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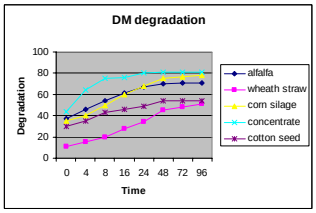
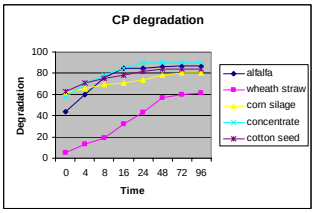
**Introduction** The *in situ* technique forms the basis of many feed evaluation systems for ruminants. Although this method is widely used, the NBT is very laborious, time-consuming, and incubations and analyses of the feed residues often last several weeks. Therefore, several other techniques have been investigated to measure and predict ruminal degradation of various chemical components of feedstuffs. The aim of this study is to investigate the possibilities of estimating *in situ* degradation characteristics of DM, CP, ADF and NDF in several feedstuffs by gas production characteristics and chemical composition.

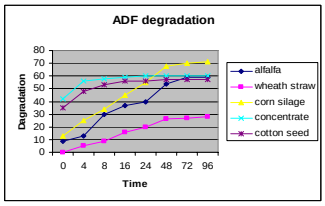
**Material and Methods** In this study we used six canulated steers ( 3 Holstein and 3 Sistanian ) with average weight 395±5 and 378±6.5 respectively. Investigations were carried out with 5 feedstuffs that included: alfalfa hay, corn silage, wheat straw, corn silage, concentrate and cotton seed. All feed samples were dried at 70 $^{C}$ and ground to pass a 1 mm screen before use. All feeds were fed at maintenance level and in two equal meals ( $8^{AM}$ and $4^{PM}$ ). The DM, CP, ADF and NDF degradability of each feedstuff were determined by incubation in nylon bags in the rumen. The experiments was carried in a completely randomized design with 6 repeat and two observation per each repeat.

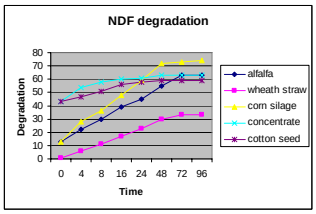
**Results** There was no significant difference in mean of DM, CP, ADF and NDF degradations between two breeds. Due to high correlation coefficient between gas production and degradation parameters, the comparison between these two methods is logical. Regression equation between amount of gas production and DM, CP, ADF and NDF degradation respectively were shown as follow:

Alfalfa: Y = 35.724 + 0.714 x , Y = 47.139 + 0.854 x, Y = 2.065 + 1.045 x, Y = 6.855 + 7.623x
Wheat straw: Y = 14.420 + 0.807 x , Y = 9.156 + 1.231 x, Y = 3.706 + 0.586, Y = 3.961 + 0.694 x
Corn silage: Y = 31.218 + 0.615 x , Y = 60.653 +0.247 x, Y = 10.678 +.0780 x, Y = 8.935 + 9.214 x Concentrate: Y = 48.351 + 0.448x , Y = 58.329 + 0.419 x, Y = 45.370 + 0.211x, Y = 45.201 + 0.240 x
Cotton seed: Y = 31.158 + 0.381 x , Y = 65.544 + 0.327 x, Y = 31.158 + 0.381 x , Y = 65.544 + 0.327 x



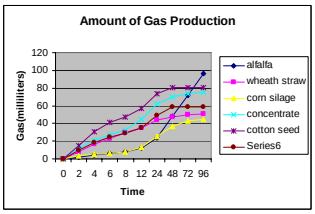




Table1-Degradation at 96 hours

| | DM | CP | ADF | NDF |
|---|---|---|---|---|
| Alfalfa | 71.52±1.33 | 86.62±1.89 | 59.51±2.21 | 63.14±2.24 |
| Straw | 51.02±1.96 | 61.29±2.21 | 28.15±1.92 | 33.09±1.45 |
| Silage | 78.89±1.73 | 80.11±2.31 | 70.74±2.05 | 74.33±2.46 |
| Concentrate | 80.59±2.16 | 90.11±2.18 | 60.09±2.21 | 63.29±2.21 |
| Cotton seed | 53.51±2.16 | 83.73±1.84 | 57.47±1.91 | 59.25±2.41 |

**Table2** Degradation parameters of DM, CP, ADF and NDF of 1=alfalfa, 2= Wheat straw, 3=corn silage, 4=concentrate and 5=cotton seed

| **feed** | a | b | c | L | P(k=0.02) | **feed** | a | b | c | L | P(k=0.02) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **1ADF** | 9.16 | 51.25 | 0.04 | 0.50 | 44.50 | **1 DM** | 36.63 | 34.43 | 0.08 | -0.10 | 64.40 |
| **2** | 0.10 | 28.19 | 0.05 | 0.30 | 20.40 | **2** | 1.54 | 41.42 | 0.04 | 1.10 | 36.70 |
| **3** | 14.66 | 56.87 | 0.05 | --- | 55.56 | **3** | 33.13 | 44.97 | 0.06 | --- | 66.43 |
| **4** | 54.17 | 6.20 | 0.08 | --- | 59.13 | **4** | 39.28 | 39.64 | 0.25 | --- | 76.03 |
| **5** | 39.95 | 17.04 | 0.17 | --- | 55.16 | **5** | 27.61 | 24.44 | 0.11 | --- | 48.16 |
| **1NDF** | 11.89 | 38.26 | 0.10 | 0.60 | 4350 | **1 CP** | 44.19 | 42.17 | 0.23 | 1.90 | 81.50 |
| **2** | 0.35 | 33.23 | 0.05 | 0.20 | 23.70 | **2** | 5.42 | 57.20 | 0.05 | 1.50 | 44.00 |
| **3** | 15.99 | 59.25 | 0.05 | --- | 58.77 | **3** | 64.14 | 16.58 | 0.04 | --- | 74.97 |
| **4** | 50.01 | 12.79 | 0.11 | --- | 60.84 | **4** | 85.27 | 32.50 | 0.12 | --- | 85.97 |
| **5** | 40.33 | 19.21 | 0.11 | --- | 56.57 | **5** | 66.82 | 17.66 | 0.07 | --- | 80.71 |

**Conclusion** The validity of rumen degradation data generated by the *in situ* nylon bag technique may be suspect considering the occasionally appreciable particle loss, particularly with starch containing feeds. By use of gas production data and its equations, we can estimate metabolisble energy of feedstuffs, without needing to fistulated animals, therefore this technique is better, Simpler and more accurate than NB method.

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# Effect of tannins in the fermentation of legume forages using a gas based method

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**Introduction** Legume forages are an important source of protein in ruminant feeding but some legumes contain anti-nutritional factors, such as tannins, which can interfere in fermentability. *In vitro* methods to determine quality of feeds are important to nutritionists. These methods are less expensive, less time consuming and allow more control of experimental conditions than *in vivo* experiments. The aim of this work was to evaluate the nutritive value of four Brazilian legume forages, with different tannin levels, using the *in vitro* bioassay technique.

**Materials and methods** Leaves of ALF – alfalfa (*Medicago sativa*), GND – feijão guandu (*Cajanus cajan*), MPT – mucuna preta (*Mucuna aterrina*) and MCZ – mucuna cinza (*Mucuna pluriens*) were used. Three samples of each plant were collected, dried in forced-air oven at 40-45ºC, for 48h and grounded to pass a 1 mm screen. Plants were chemically characterized for dry matter – DM, organic matter – OM, crude protein – CP, neutral- and acid-detergent fibre – NDF and ADF,, total phenols – TP, total tannins – TT, and condensed tannins – CT. The bioassay technique was measured following Cabral Filho et al. (2005) and Makkar et al. (1993). It was used a semi-automatic gas production system. The rumen contents were obtained from three adult male sheep via rumen cannula prior to the morning feeding. This content was used as inoculum and the samples (1g DM) were incubated at 39ºC for 24h. Substrates were incubated, in duplicate, with or without 1g of polyethylene glycol (PEG MW 6000), after 24h the substrates were filtered and determinate the rumen degradability DM. A factorial design (4 plants x 2 treatments x 4 inocula) was used to analysis of variance and means were compared using Duncan test (SAS, 2000).

**Results** The chemical characterization regarding the contents of CP, NDF and ADF suggests that these four legumes had potential nutritive value. MCZ showed the highest contents of TT, CT and TP, and ALF the lowest (Table 1). ALF showed the higher CP contents and the lowest NDF content, whereas GND had the inverse values. Gas production increase in presence of PEG was significantly lower ($P<0.01$) for ALF than for other legumes, which were not different among them ($P>0.05$). There was a correlation between PEG effect and TP and TT, but not for PEG effect and CT ($r = 0.498$; 0.504; 0.434 and $P = 0.0496$; 0.0468; 0.0943, respectively). No differences ($P>0.05$) were observed for partitioning factor, even analyzing with or without PEG, separately. ALF showed the highest DMD24.

**Table 1.** Chemical composition, increase in gas production, using PEG as tanning attenuating agent, and partitioning factor (PF, as ratio between gas production and OM digested) of Brazilian legume forages

| composition | feeds | | | | s.e.m. |
|---|---|---|---|---|---|
| | ALF | GND | MCZ | MPT | |
| DM (g/kg) | 947[a] | 948[a] | 943[a] | 947[a] | 25.3 |
| OM (g/kg DM) | 918[b] | 950[a] | 926[b] | 932[ab] | 7.2 |
| CP (g/kg DM) | 184[a] | 143[b] | 156[b] | 163[ab] | 6.8 |
| NDF (g/kg DM) | 606[b] | 689[a] | 633[ab] | 665[ab] | 21.2 |
| ADF (g/kg DM) | 364[a] | 493[a] | 556[a] | 464[a] | 62.2 |
| TP (eq-g tannic acid/kg DM) | 10.3[c] | 29.5[b] | 51.3[a] | 24.0[b] | 2.94 |
| TT (eq-g tannic acid /kg DM) | 6.4[c] | 21.1[b] | 34.7[a] | 16.4[b] | 2.47 |
| CT (eq-g leucocyanidin/kg DM) | 0.2[c] | 21.4[b] | 49.6[a] | 7.3[c] | 2.62 |
| Increase in gas production (%) | 1.4[b] | 8.7[a] | 12.1[a] | 14.1[a] | 1.91 |
| PF with PEG (ml/mg OMD*) 24h | 5.29[a] | 4.66[a] | 4.77[a] | 4.92[a] | 0.388 |
| PF without PEG (ml/mg OMD*) 24h | 5.17[a] | 4.89[a] | 4.62[a] | 4.94[a] | 0.388 |
| DMD24 (g/kg)** | 0.335[a] | 0.270[b] | 0.289[b] | 0.290[b] | 0.0105 |

[a,b,c] means followed by distinct superscripts, within rows, are significantly different (Duncan test, $P<0.05$); * OMD: organic matter digested, estimated from gas production assay; ** DMD24: dry matter degradability after 24h of incubation

**Conclusions** All tested feeds presented high protein levels. GND, MCZ and MPT contained anti-nutritional factors (tannins), which did not affect rumen fermentability, and all feeds can be used in ruminant nutrition.

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# Feed intake and digestive responses of dairy cow fed lucerne hay varying in particle size

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**Introduction** Chemical composition and physical characteristics of diet influences its optimal utilization in dairy cow. Neutral detergent fiber measures the chemical characteristics, but not the physical characteristics of fiber such as particle size and density (Mertens, 1997). Both the amount and physical form of fiber are important in the diet of lactating dairy cow for maintaining normal milk fat content, chewing and digestive activities. A sufficient supply of long particles or NDF must be included in the ration to increase chewing activities, maintaining the rumen pH and providing an optimal rumen environment in order to avoid metabolic disorders. Current NRC guidelines (NRC, 2001) have proven useful information in defining animal requirements and feed composition but do not provide detailed recommendation on physical form of ration. The objective of this experiment was to determine the effect of lucerne particle size on feed intake and digestibility in dairy cow in early lactation period.

**Materials and methods** Eight primiparous and multiparous Holstein cows averaging days in milk of 28 ±10 and body weight of 539 ± 11 were allotted to a 4×4 Latin square design in early lactation phase . The experiment consisted of an adaptation period of 10 days followed by a collection period of 5 days. The chemical composition of all diets was similar but varied in particle size distribution or geometric mean (GM) of particles. Diets contained Lucerne hay (27.5), corn silage (7.5), barley grain (20.5), corn (9.5), soybean meal (7.5), cottonseed meal (6.5), cottonseed (10), wheat bran (3.5), beet pulp (3.3), tallow (2.5), limestone (0.6), salt (0.3) and mineral and vitamin premix (0.5) %(of DM). Diets were formulated according to the NRC recommendation (2001). Four particle sizes of lucerne hay including 5, 10, 15 and 20 mm with the respective geometric means of 2.89, 3.15, 3.94 and 4.62 mm, were prepared by a fodder chopper. Cows were housed in a tie-stall and fed *ad libitum*, twice daily at 0900 and 1800 h. The daily DMI was measured for all cows. Forage, TMR and ort samples were taken. Acid insoluble ash (AIA) was used as internal marker for digestibility measurements.

**Results** Nutrient digestibilities, voluntary DMI and the supplied nutrients are shown in Table1. Dry matter and nutrient intakes were not affected by reducing particle sizes. Dry matter digestibility decreased significantly ($p<0.05$) with reduction in particle sizes.

**Table1** Intake and nutrient digestibilities of the experimental diets fed to the cow in TMR form.

| Item | Diet | | | | SEM | P [1] |
|---|---|---|---|---|---|---|
| | 5 | 10 | 15 | 20 | | |
| Intake(kg/d) | | | | | | |
| DM | 23.95 | 23.75 | 23.69 | 23.45 | 0.43 | NS |
| OM | 21.96 | 21.77 | 21.71 | 21.50 | 0.40 | NS |
| NDF | 8.02 | 7.94 | 7.91 | 7.83 | 0.14 | NS |
| $peNDF_{>1.18}$ [2] | 6.02 | 6.12 | 6.32 | 6.35 | 0.11 | NS |
| ADF | 4.86 | 4.81 | 4.79 | 4.74 | 0.09 | NS |
| Digestibility (%) | | | | | | |
| DM | $62.33^{c}$ | $62.96^{b}$ | $63.35^{a}$ | $63.38^{a}$ | 0.07 | * |
| OM | 75.28 | 75.95 | 75.30 | 75.99 | 0.50 | NS |
| NDF | 53.66 | 52.37 | 53.61 | 54.05 | 4.52 | NS |
| ADF | 44.10 | 39.36 | 44.55 | 43.71 | 5.03 | NS |
| NFC | 82.41 | 83.65 | 82.44 | 81.69 | 2.40 | NS |

[a,b,c] Means within a row with different superscripts differed significantly ($p<0.05$).
[1] quadratic effect
[2] $peNDF_{>1.18}$= Physically effective NDF based on DM retained on 1.18 sieve (Mertens, 1997).
NS=Non significant; *$p\leq0.05$.

**Conclusions** Results of the present study showed that the reduction in particle size led to low DM digestibility ($p<0.05$) but had no significant effect on digestibility of OM, NDF, ADF and NFC. Digestibility in ruminants is a result of competition between ruminal retention time for digestion and ruminal passage rate (van soest, 1994). Therefore, the reduction of DM digestibility as a result of reduction in particle sizes could be due to reducing retention time or increasing ruminal passage rate .

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# Ruminal peptide and ammonia nitrogen concentrations in steers fed diets with different concentrate to lucerne hay ratios

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**Introduction** In ruminants, as much as 50% of the dietary crude protein can be converted to ammonia by ruminal microorganisms. A part of ammonia can be utilized as a bacterial nitrogen source; however, rates of ammonia production often exceed rates of ammonia utilization. Peptides are intermediates in the conversion of ingested protein to ammonia in the rumen and their accumulation depends upon the nature of diet (Mesgaran & Parker, 1995). The objective of the present experiment was to investigate the effect of diets differing in concentrate: lucerne hay ratios on the ruminal pH, ammonia-nitrogen concentration and ruminal peptide nitrogen concentration in Holstein steers.

**Materials and methods** Four Holstein steers with initial body weight of 300±15 kg fitted with ruminal Fistulae were used in a 4×4 Latin square design (28 days of each period). Animals were fed 7 kg of DM of diets differing in concentrate [155 g CP $kg^{-1}$ of DM; consisted of maize, barley, soybean meal, sugar beet pulp, wheat bran, cottonseed meal, $CaCo_3$, mineral and vitamin premix, salt (30, 34, 8, 5, 10, 12, 0.3, 0.5, and 0.2 g/100g DM; respectively)] to lucerne hay ratios as 60:40 ($C_{60}$:$L_{40}$), 70:30 ($C_{70}$:$L_{30}$), 80:20 ($C_{80}$:$L_{20}$) and 90:10 ($C_{90}$:$L_{10}$). Steers fed the experimental diets as total mixed ration twice daily at 0800 and 2000 h. At day 24 of the each experimental period, ruminal fluid samples were collected, by suction, before the morning feeding, 4 and 6 h post feeding. The pH of the ruminal fluid samples was measured immediately with a portable pH meter (Metrohm 744). Ruminal fluid samples were prepared for peptide-N using sulphate-tungstate method described by Chen *et al.* (1987). The percoloric and tugstate acid-precipitates nitrogen were assayed by a standard macro-Kjeldahl procedure. For samples designated for $NH_3$ analysis, 10 ml of ruminal fluid from each collection were acidified with 10 ml of 0.2 N HCl. Samples were analyzed for $NH_3$-N using distilation method (Kjeltec 1030 Analyzer tecator). Data were analyzed using the GLM procedure of SAS (y = Mean + Treatment + Animal + Period + Time + Time × Treatment + residual) and the means compared by the Duncan test ($P<0.05$).

**Results** The ruminal pH and ammonia-N and peptide-N concentrations at different sampling time are shown in the Table 1. Results indicated that the ruminal peptide-N was not significantly influenced by the diets and sampling time. Ruminal pH and ammonia-N concentration decreased from 6.54 ($C_{60}$:$L_{40}$) and 17.95 ($C_{70}$:$L_{30}$) to 5.87 ($C_{90}$:$L_{10}$) and 11.44 ($C_{90}$:$L_{10}$), respectively, ($P<0.05$). ruminal pH and ammonia-N concentration were significantly affected by the treatments and sampling time ($P<0.05$).

**Table 1** The ruminal pH, and ammonia-N and peptide-N concentrations (mg/dl) in the rumen fluid of Holstein steers fed diets differing in concentrate: lucerne hay ratios

| Item | Time (h) | Concentrate: lucerne hay ratio | | | | Treatment effect | | Time effect | |
|---|---|---|---|---|---|---|---|---|---|
| | | 60:40 | 70:30 | 80:20 | 90:10 | SEM | *P* | SEM | *P* |
| pH | 0.0 | 6.91 | 6.76 | 6.52 | 6.50 | 0.09 | 0.01 | 0.07 | 0.01 |
| | 4.0 | 6.30 | 6.20 | 5.94 | 5.50 | | | | |
| | 6.0 | 6.43 | 6.51 | 6.21 | 5.63 | | | | |
| $NH_3$-N | 0.0 | 12.48 | 19.48 | 15.75 | 12.97 | 1.54 | 0.03 | 1.33 | 0.01 |
| | 4.0 | 18.87 | 19.98 | 13.87 | 15.42 | | | | |
| | 6.0 | 13.74 | 14.41 | 10.52 | 5.94 | | | | |
| peptide-N | 0.0 | 5.67 | 4.92 | 1.49 | 0.18 | 1.30 | 0.15 | 1.12 | 0.98 |
| | 4.0 | 2.07 | 0.13 | 0.07 | 8.97 | | | | |
| | 6.0 | 6.95 | 3.23 | 0.24 | 1.66 | | | | |

**Conclusions** Results showed the ruminal peptide-N concentration typically increased at 6 hours after feeding. When animals fed a high concentrate: lucerne hay ratios ($C_{80}$:$L_{20}$), ruminal peptide-N concentration was significantly lower than those fed $C_{60}$:$L_{40}$ and $C_{70}$:$L_{30}$. The results of the present study demonstrated that the increasing of concentrate in diets caused to decrease the ruminal pH and ammonia-N concentration. The increasing of concentrate may reduce proteolysis in the rumen. Furthermore, provided ruminal energy facilitates microbial yield and the demand for ruminally available nitrogen. Ruminal pH decreased continuously until 4 h after feeding. In overall, the results of the present experiment suggested that the diets containing higher concentrate had a significant effect on ruminal pH and ammonia-N concentration.

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# Physically effectiveness of beet pulp in dairy cows 1: physical characteristics, ruminal kinetics of nutrients degradation, hydration, and functional specific gravity

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**Introduction** Some physical properties including particle size, functional specific gravity (FSG; Teimouri Yansari et al., 2004), hydration rate, water holding capacity (WHC) and ionion-cation exchange (Van Soest, 1994) influenced on physically effective factor (pef), but only particle size measurement is central to all effective fibre systems. The objective of this study was to evaluate the effects of three types of beet pulp (BP) on physical characteristics including bulk density, particle size, kinetics of hydration, FSG, WHC, and intrinsic osmotic pressure that measured using *in vitro* and *in situ* methods.

**Materials and methods** The bulk density, WHC, feed solubilization intrinsic osmotic pressure of three types fine (FBP), normal (NBP) and pelleted (PBP) of BP (PBP; Giger-Reverdin, 2000) and particle size distribution were determined using PSPS (Kononoff, 2002). Using McDougal's buffer, the kinetic of hydration, FSG, and changes of FSG were measured. The ruminal nutrients degradation and kinetic of hydration, FSG, and changes of FSG of samples of three types including was determined *in situ*, using two cannulated Holstein steers (1 yr old, BW= 335.2± 10.3 kg; Ørskov and MacDonald, 1979). The two bags were incubated in the rumen for 0, 0.5, 1, 1.5, 3, 6, 12, 18, 24, 36, and 48 h. After incubation in rumen and removal of bags, the kinetic of hydration, FSG, and changes of FSG of samples measured with 100 ml pycnometers (Wattiaux, 1990), without washing. Data were analysed by using the GLM and MIXED procedure of SAS® (1998) and differences between treatments were separated by Duncan at alpha =0.05).

**Results and Discussion**

**Table1**. Summary of results[1]

| Item | Three types of beet pulp | | | SEM | P-Value |
|---|---|---|---|---|---|
| | Fine | Normal | Pelleted | | |
| DM | 94.36 | 94.35 | 94.62 | 2.25 | NS |
| CP | 9.61 | 9.69 | 9.58 | 0.41 | NS |
| NDF | 39.67 | 40.12 | 39.68 | 0.54 | NS |
| ADF | 23.88 | 23.91 | 24.21 | 0.31 | NS |
| EE | 0.78 | 0.82 | 0.83 | 0.04 | NS |
| Ash | 7.31 | 7.45 | 8.44 | 0.4 | NS |
| NFC | 42.63 | 44.92 | 41.47 | 0.28 | NS |
| Geometric mean of particle size | $1.22^{b}$ | $3.26^{a}$ | …. | …. | *** |
| SD of Geometric mean | 1.91 | 2.01 | …. | …. | NS |
| Physically effective factors | $0.26^{b}$ | $0.88^{a}$ | …. | …. | ** |
| Bulk density50 [2](g/ml) | $0.767^{b}$ | $0.625^{c}$ | $0.864^{a}$ | 0.001 | * |
| Bulk density100[2] (g/ml) | $0.770^{b}$ | $0.630^{c}$ | $0.872^{a}$ | 0.0007 | * |
| Functional specific gravity | $1.416^{a}$ | $1.371^{c}$ | $1.384^{b}$ | 0.002 | * |
| Water holding capacity (g/DM) | $4.318^{c}$ | $5.261^{a}$ | $4.881^{b}$ | 0.002 | ** |
| Hydration rate (g/DM/Min) | $0.0527^{b}$ | $0.0663^{a}$ | $0.0657^{a}$ | 0.007 | *** |
| Soluble DM (%) | $28.61^{a}$ | $17.98^{c}$ | $23.66^{b}$ | 0.176 | *** |
| Soluble ash (%) | $45.18^{a}$ | $37.79^{b}$ | $39.36^{b}$ | 0.524 | ** |
| Osmotic pressure (mOsm/kg H2O) | $47.87^{a}$ | $45.36^{b}$ | $45.49^{b}$ | 0.227 | ** |
| Degradability of dry matter | | | | | |
| Soluble fraction (%) | $31.00^{a}$ | $25.33^{b}$ | $22.67^{b}$ | 0.005 | *** |
| Slowly digestible fraction (%) | $59.00^{a}$ | $57.00^{ab}$ | $55.66^{b}$ | 0.004 | * |
| Fractional rate of disappearance (%/h) | $0.112^{a}$ | $0.103^{b}$ | $0.101^{b}$ | 0.006 | * |
| *Effective degradability (%/h) at* Kp = 0.05 | $71.75^{a}$ | $63.66^{b}$ | $59.90^{c}$ | 0.002 | ** |
| Degradability of NDF | | | | | |
| Soluble fraction (%) | $28.00^{a}$ | $25.00^{b}$ | $22.00^{c}$ | 0.003 | ** |
| Slowly digestible fraction (%) | $58.00^{a}$ | $54.00^{b}$ | $53.00^{b}$ | 0.002 | *** |
| Fractional rate of disappearance (%/h) | $0.098^{a}$ | $0.088^{b}$ | $0.084^{c}$ | 0.001 | *** |
| *Effective degradability (%/h) at* Kp = 0.05 | $66.40^{a}$ | $59.43^{b}$ | $55.22^{c}$ | 0.003 | *** |
| Degradability of CP | | | | | |
| Soluble fraction (%) | $21.00^{a}$ | $18.00^{b}$ | $14.00^{c}$ | 0.002 | ** |
| Slowly digestible fraction (%) | $66.00^{a}$ | $65.00^{a}$ | $63.00^{b}$ | 0.002 | *** |
| Fractional rate of disappearance (%/h) | $0.108^{a}$ | $0.100^{b}$ | $0.097^{c}$ | 0.001 | *** |
| *Effective degradability (%/h) at* Kp = 0.05 | $66.16^{a}$ | $61.29^{b}$ | $55.64^{c}$ | 0.002 | *** |
| Ruminal kinetics of hydration | | | | | |
| Initial water content (g/ g insoluble DM) | 0.062 | 0.064 | 0.064 | 0.051 | |
| Rate oh hydration (g/ g insoluble DM/ h) | $0.065^{b}$ | $0.075^{a}$ | $0.064^{b}$ | 0.002 | ** |
| Water holding capacity (g/ g insoluble DM) | $3.25^{c}$ | $4.21^{a}$ | $3.87^{b}$ | 0.021 | *** |

[1]Means within a row with different subscripts differ ($P<0.05$).NS, Not significant;$^{*}P \leq 0.05$,$^{**}P \leq 0.01$,$^{***}P \leq 0.001$.

**Conclusion** The physical methods gave new information about the nutritive value of feedstuffs for ruminant and they can be used to differentiate between feedstuffs and taken into account in feed formulation. They might explain part of the role played by rumen or on feedstuffs, which is not taken into account by the chemical approach. Grinding and peletting hay alters the physical characteristics of the BP and, consequently, may affect the time that cows spend chewing. As beet pulp has lower than critical size, can easily pass from the riticulo-rumen orifice, therefore, its functional specific gravity is more important to control ruminal retention time and degradation.

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# Measurement of microbial protein synthesis in Iranian buffalo rumen (Mazandran Province) by purine derivatives excretion method

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**Introduction** Rumen microbes are rich in nucleic acid: around 18% of total nitrogen is present on nucleic acids or 11% in purines. Rumen microbes constitue the major source of protein supply to the ruminant. The purines from the rumen microbes are metabolized and excreted in the urine as their end products: hypoxanthine, xanthine, uric acid and allantoin. In buffalo and cattle because of high xanthine oxidase activity in intestine and blood, hypoxanthine and xanthine convert to uric acid therefore only uric acid and allantoin excreted in urine way (Chen, X. B., Ørskov, E. R., 2003). This research carried out to use excretion of purine derivatives namely allantoin and uric acid as a parameter to estimate the microbial protein synthesis in the rumen of native swamp buffalo in north of iran, Mazandaran Province.

**Materials and methods** In this experiment four Iranian male swamp buffalo with the average live body weight of 140±10 kg were used. animals were housed in four metabolic cages and fitted with urine collection instrument. It has been experimented four diets consist of 0 % ( all forage diet), 15%, 30% and 45% concentrate in a latin square design, in four periods. Each period lasted 21 days (11 days foe adaptation and 10 days for urine collection). Diets was offered twice daily at 8:00 am and 4:00 pm, in two equal meals. Urine was collected in a container each days. To prevent microbial degradation of purines, urine was acidified by 10% $H_2SO_4$ to a pH of 2-3. urine was diluted by distilled water to prevent the precipitation of purine derivatives during the storage period. A subsample of 40 ml was took and stored in -20$^{oc}$ for further experiment.

**Results** The results of this experiment indicated that increasing concentrate level in diet of swamp buffalo was affected on the amount of PD excreted in urine. The amount of allantoin excreted in urine was under affect of increasing concentrate level form 0% to 45%. It increased from 13.2 to 21.8 mmol/day and a significant difference was observed (P<0.01). there was no significant difference among uric acid excretion from buffalos fed (15% to 30%) and (30% t0 45%) concentrate level, but it was significant between 45% and 15% concentrate level (P<0.01). the difference between control diet and other diets containing different level of concentrate (15%, 30% and 45%) was significant (P<0.01). total purine derivatives excretion and microbial protein synthesis in rumen significantly affected byincreasing concentrate and increase from 14.5 in control diet to 18.11, 21.58 and 24.55 mmol/day and from 38.76 in control diet to 60.44, 81.48 and 99.43 g/day in buffalos fed diets containing 15%, 30% and 45% respectively (table 1).

**Table 1** microbial nitrogen synthesis measured in rumen of Iranian buffalo (Mazandaran Province)

| Purine derivatives | Diets | | | | |
|---|---|---|---|---|---|
| | 45% concentrate | 30% concentrate | 15% concentrate | 0% concentrate | SE |
| Allantoin (mmol/d) | 21.79 ± .73 $^{a}$ | 19.15 ± 1.44 $^{b}$ | 15.84 ± 1.26 $^{c}$ | 13.21 ± .31 $^{d}$ | 0.94 |
| Uric acid (mmol/d) | 2.75 ± .30 $^{a}$ | 2.44 ± .12 $^{ab}$ | 2.28 ± .22 $^{bc}$ | 1.326 ± .16$^{d}$ | 0.10 |
| Total PD (mmol/d) | 24.55 ± .96 $^{a}$ | 21.59 ± 1.50 $^{b}$ | 18.11 ± 1.44 $^{c}$ | 14.54 ± .36 $^{c}$ | 1.06 |
| Microbial nitrogen synthesis (g/d) | 99.43 ± 5.02 $^{a}$ | 81.48 ± 7.36 $^{b}$ | 60.44 ± 7.99 $^{c}$ | 38.76 ± 2.49 $^{d}$ | 5.73 |

Values in table are mean ± SD (P<0.01)

**Conclusions** The results of this experiment indicated that increasing the concentrate level in diet of swamp buffalo was increased the amount of microbial protein synthesised in rumen. the reason can be related to increasing the amount of digestible organic matter fermented (DOMR) in rumen, as a result of increasing concentrate to forage ratio. Based on ARC(984) the amount of microbial protein synthesis in rumen is 32 g/kg DOMR. Therefore with increasing DOMR, microbial protein synthesis will be increased. On the other hand, with increasing concentrate proportion, OM digestibility of diet will be increased too.

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# Evaluation of nutritive value of tropical clover (Trifolium resupinatum) using gas production technique

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**Introduction** Gas measurement provides a useful data on digestion kinetics of both soluble and insoluble fractions of feedstuffs (Getachew *et al.* 1998). Carbohydrate portion of each feed is divided into three digestible fraction: the A fraction, containing sugars, short oligosaccharides, and organic acids; the $B_1$ fraction, containing starch and pectin; and $B_2$, the digestible fibre fraction (Doane *et al*. 1998). The objective of this study was to measure of fermentation parameters of tropical clover (Trifolium resupinatum) (pre bud, full bud, pre bloom and full bloom) using gas production technique.

**Materials and methods** The tropical clover samples were chopped to 2 cm length. Treatments contain PB: pre bud, FP: full bud, PBL: pre bloom, and FBL: full bloom. Three sheep (38±1.5 kg) used. The sheep were fed a diet consisting of 600 g $kg^{-1}$ concentrate and 400 g $kg^{-1}$ forage containing DE (3.35 Mcal/kg DM) and CP (160 g/kg DM) and were used as donors of ruminal fluid for preparation of inoculums. Approximately 300 mg of feed was weighted and placed into a serum bottle. Buffered rumen fluid with McDougal buffer (20 ml) was pipetted into each serum bottle. All the serum bottle incubated for 2, 8, 12, 24 and 48h (3 bottle for each time). Gas production was measured by Fedorak and Hrudey (1983) method. Since the accumulation of gas production was measured, so for determining of the effects of cut stage on digestion kinetics, the gas production data at the different times was fitted with equation of $p=a+b\ (1-e^{-ct})$ that p is gas production in time t, a + b is potential of gas production, c is rate of gas production of insoluble fraction and t is time. . The data was analyzed using the ANOVA procedure of SAS (SAS Institute 1987).

**Results** The results are shown in Table 1. The results indicated that gas production of soluble and insoluble of FB (a+b) was rather than the other treatments ($P<0.05$). The rate of gas production (c) of FBL was the highest ($P<0.05$). The results showed with promoting of growth increased the rate of gas production, but decreased the potential gas production. Regarding to the differences in chemical composition in different stages cut of clover, the obtained results can be predicted.

**Table 1** Gas volume production of treatments at the times of incubation (ml/g)*

| | Time of incubation (h) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 2 | 8 | 12 | 24 | 48 | (a +b)[1] | c[2] |
| PB | 11.1[d] | 115[c] | 158[b] | 210[b] | 240[b] | 232.7[b] | 0.08[d] |
| FB | 16[a] | 150[a] | 190[a] | 235[a] | 270[a] | 268.7[a] | 0.09[c] |
| PBL | 15[b] | 135[b] | 160[b] | 190[c] | 215[c] | 225.6[c] | 0.1[b] |
| FBL | 14.5[c] | 90[d] | 110[c] | 115[d] | 124[d] | 121.5[d] | 0.11[a] |
| SEM | 0.54 | 0.45 | 0.5 | 0.48 | 0.58 | 0.59 | 0.001 |
| $R^2$ | - | - | - | - | - | 0.99 | 0.99 |

1- The potential of gas production
2- The rate of gas production
* The means within a column without common letter differ ($p<0.05$)

**Conclusions** There was significant difference in fermentation characteristics of clover with different cut stages that should be considered in ration formulation of ruminants.

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# Evaluation of some ruminant feedstuffs using gas production technique, *in vitro*

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**Introduction** In Egypt, animals suffer from under feeding and mal-nutrition due to the shortage of local produced feed which are not sufficient to cover the nutritional requirements of animals. The annually amount produced of agro-by-products in Egypt are around 2.5 million ton of rice straw and one million ton of sugarcane bagasse. These wastes are usually burned causing environmental pollution. The potential use of these wastes in ruminant ration will participate in reducing the shortage of feedstuffs and subsequently increase milk and meat production in Egypt. Gas measuring technique has been widely used for evaluation of nutritive value particularly to estimate agro-industry by-products, different feed classes and energy value of straws. Therefore, the main objective of this study was to assess the nutritive value of rice straw, date stone, sugarcane bagasse and berseem hay using the *in vitro* gas production technique.

**Materials and methods** Rice straw, linseed straw, date stone, sugarcane bagasse and berseem (*Trifolum alexandrinum*) hay were ground to chemical analysis and *in vitro* gas production measurements. Rumen contents were collected before the morning feeding from three rumen-cannulated sheep fed on berseem hay and commercial concentrate mixture diet. Rumen fluid was mixed with buffer solution (1:2 v/v), flushed with $CO_2$ and maintained in a water bath at 39 ºC. Samples (200±10 mg) of air-dry feedstuffs were accurately weighted into syringe fitted with plungers. Buffered rumen fluid (30 ml) was pipetted into each syringe, containing the feed samples, and the syringes were immediately placed into the water bath at 39 ºC. The gas production was recorded after 3, 6, 9, 12, 24, 48, 72, and 96 h of incubation. Total gas values were corrected for the blank incubation, and reported gas values are expressed in ml per 200 mg of DM. Cumulative gas production was fitted iteratively to the exponential model. The energy value of forages can be calculated from the amount of gas produced at 24 h of incubation with supplementary analysis of crude protein, ash and ether extract. Data were subjected to analysis of variance (ANOVA) using the General Linear Model.

**Results** The chemical analysis of these roughages showed that sugarcane bagasse contained less ash (21.0 g/kg) and protein (12.9 g/kg) but ash content in linseed straw was very high (259.5 g/kg). Sugarcane bagasse and linseed straw have the highest crude fibre content in comparison to other feedstuffs. Date stone contained the lowest crude fibre value. The NDF and ADF were significantly different among roughages used in this study. The linseed straw, rice straw and sugarcane bagasse showed the highest levels of NDF and ADF. Kinetics of gas production obtained from the exponential model were ($P<0.05$) significant differences among roughages (Table 1). The predicted metabolizable energy (ME) and net energy (NE) profile were high in berseem hay and sugarcane bagasse. Linseed straw had significantly low values of ME and NE. The data showed that there was no significant difference among rice straw, sugarcane bagasse and date stone.

**Table 1** Cumulative gas production and kinetic parameters[(1)] of roughages incubated with rumen fluid *in vitro*

| Parameter[(2)] | Roughages | | | | | SED |
|---|---|---|---|---|---|---|
| | Berseem hay | Rice straw | Linseed straw | Date stone | Sugarecane bagasse | |
| A (ml/200 mg) | 50.92 [a] | 38.90 [b] | 14.98 [c] | 46.69 [a] | 39.46 [b] | 2.57 |
| b ($h^{-1}$) | 0.035 [b] | 0.055 [ab] | 0.064 [a] | 0.047 [ab] | 0.059 [a] | 0.022 |
| c ($h^{-1/2}$) | 0.1174 [a] | 0.0053 [b] | -0.0855 [b] | -0.1141 [b] | -0.0403 [b] | 0.128 |
| T (h) | 1.55 [a] | 0.59 [ab] | 0.49 [ab] | 1.50 [a] | 0.15 [b] | 0.545 |
| µ24 ($h^{-1}$) | 0.0155 | 0.0123 | 0.0152 | 0.0164 | 0.0101 | 0.004 |

[(1)] France *et al.,* (1993), [(2)] A: potential gas production; b and c: fermentation rate constants; T: lag time; µ24: fractionation rate at 24 h of incubation, [a, b, c] means followed by distinct superscripts, within rows, are significantly different (Tukey test; $P < 0.05$).

**Conclusion** The *in vitro* gas production technique can be used to determine the nutritive value of the roughages and to identify differences among their potential digestibility and energy contents. Chemical composition can be considered useful indicators for the preliminary evaluation of the likely nutritive value of feedstuffs. Date stone and sugarcane bagasse revealed that these by-products could be interesting alternative animal feed sources.

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# Effect of phenolic compounds in oak leaves (Quercus spp.) and PEG on gas production technique in sheep

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**Introduction** Oak trees (*Quercus* spp.) are the main source of animal feedstuff in the forest areas of Zagros mountain chain in Iran. The leaves of oak contain high level of tannins particularly hydrolysable, which causes mortality in ruminant animals (Makkar, 2003). Polyethylene glycol (PEG) can form a stable complex with tannins, preventing the binding between tannins and dietary proteins (Makkar, 2003). Little information is available on the nutritive value of Quercus species (*Q. persica and Q. infectoria)*. This study, therefore, is evaluating the chemical composition, phenolic compounds, organic matter digestibility (IVOMD) and metabolisable energy (ME) of these species with or without PEG using gas production technique in sheep.

**Materials and methods** Chemical composition (AOAC, 1990) and phenolic compounds (Makkar, 2000) for oak leaves were determined. Dried and ground (1mm) samples of Quercus species were incubated using the *in vitro* gas method of Menke and Steingass (1988). Each syringe contained 375 mg of dried sample without (control) and with 750 mg of PEG 6000. Triplicate samples for each treatment were used. Gas volume was read at 2, 4, 6, 8, 12 and 24 h. After 24 h incubation, gas production was recorded. Feed organic matter digestibility (OMD) and metabolisable energy (ME) were estimated from equations of Menke and Steingass (1988). Data on chemical and phenolic compounds, *in vitro* gas production and digestibility estimates were statistically analysed using ANOVA. When the interaction between species and PEG in gas production was significant, the differences between control and PEG within oak species were examined by using T test.

**Results** The chemical composition and phenolic compounds for the oak species is shown in Table 1. *Quercus infectoria* leaves have lower organic matter (OM), crude protein (CP), but contain higher and lignin in comparison to *Q. persica*. In both Quercus spp, the level of hydrolysable tannins (HT) is high. *Q. infectoria* species has higher total phenols (TPH), total tannin (TT), condensed tannin (CT) and HT than those in *Q. persica.*

**Table 1** Chemical composition and tannin fractions (g $kg^{-1}$ DM) of leaves from two different oak species.

| | DM | OM | CP | NDF | Lignin | TPH | TT | CT | HT |
|---|---|---|---|---|---|---|---|---|---|
| *Q. persica* | 939 | 951 | 115 | 532 | 98 | 78 | 73 | 14 | 46 |
| *Q. infectoria* | 943 | 927 | 92 | 540 | 103 | 115 | 109 | 15 | 87 |

Means in the same row with different superscripts (a-c) differ ($p<0.01$). * S.E.M.: standard error of the means.

Gas production characteristics are given in Table 2. The potential gas production (a + b), ME and OMD in *Q. persica* was significantly ($p<0.01$) higher than in *Q. infectoria*. In both species, the addition of PEG has significantly ($p<0.01$) increased Gas production characteristics b, (a+b), OMD and ME, however, these parameters were improved more significantly ($p<0.01$) in *Quercus persica* than those in *Q. infectoria.*

**Table 2** Gas production characteristics (ml/200 mg DM), metabolisable energy (MJ/kg DM) and organic matter digestibility (g/kg DM) in oak species treated (+PEG) and without PEG (control) (n= 3)

| | Q. persica | | Q. infectoria | | | | | |
|---|---|---|---|---|---|---|---|---|
| | control | +PEG | control | +PEG | SEM | S | P | S*P |
| a | 1.56 | 1.26 | 1.69 | 1.74 | 0.092 | ** | NS | NS |
| b | 28.1 | 33.5 | 27.6 | 33.1 | 0.106 | ** | ** | NS |
| a+b | 29.6 | 34.7 | 29.5 | 34.9 | 0.133 | NS | ** | NS |
| c | 0.047 | 0.053 | 0.051 | 0.053 | 0.001 | NS | ** | NS |
| ME | $6.0^{b}$ | $6.4^{a}$ | $5.6^{b}$ | $6.3^{a}$ | 0.022 | ** | ** | * |
| OMD | 420 | 459 | 405 | 458 | 0.247 | ** | ** | NS |

Means with different letters within each species are significantly different; NS: non significant. * $P<0.05$; ** $P<0.01$; S: species; P: PEG; S*P: interaction between S and P.

**Conclusions** The differences in the cell-wall fraction, TPH, TT, CT and HT between species were not parallel with the gas production characteristics. However, OMD and ME were improved due to the inclusion of PEG. Based on the results obtained from PEG treatment and CP levels, these species have potential as livestock fodder.

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# Effects of wood ash, calcium hydroxide and polyethylene glycol treatments on the nutrient availability and on the biological activity of tannins using gas production technique

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**Introduction** Phenolic compounds, mainly tannins, depress the nutritive value of many feedstuffs. Tannins are hydrosoluble polymers which form complexes, essentially with proteins. These complexes are broken under conditions of high acidity (pH <3.5) or high alkalinity (pH >7.5). The aim of this work was to investigate the effects of different treatments on chemical composition and tannin bioassay of tanniniferous plants from the Northeast of Brazil.

**Materials and methods** The selected browses were ANG – angico (*Anadenanthera macrocarpa*), ARO – aroeira (*Astronian urundeuva*), JUR – jurema preta (*Mimosa hostilis*), MAL – malva branca (*Sida cordifolia*) and MAN – maniçoba (*Manihot pseudoglaziovii*). The leaf samples were collected, sun-dried for 48h and grounded to pass a 1 mm screen. These browses were treated with polyethylene glycol (PEG 4000 – Vetec)) solution (2g PEG/g condensed tannin present in the plants), wood ash (10%) and calcium hydroxide (10%). The PEG solution was sprayed onto the samples and allowed to react for 30 min before proceeding to tannin analysis. Solutions of ashes or calcium hydroxide were used for soaking the samples during 6 h, before proceeding to tannin analysis. Plants were chemically characterized following AOAC (1995) and Makkar (2000). The bioassay technique was used following Cabral Filho et al (2005). The inoculum was obtained from three adult male sheep via their rumen cannula prior to the morning feeding and samples were incubated at 39ºC for 24h. with or without 1g PEG. A factorial design was used and means were compared by Dunnett test using SAS system (SAS, 2000).

**Results** There was a wide variation among the browses chemical com-position and total phenol (TP), total tannins (TT) and condensed tannins (CT) (Table 1). The treatments were decreased the CP (P< 0.05) in ANG, ARO and MAL but increased the CP content in JUR and MAN. NDF content was declined (P<0.05) in JUR with PEG or wood ash treatments and MAL but increased (P<0.05) in MAN with all treatments. The treatments were significantly decreased CT in all investigated browses. The highest proportional increase in gas production with addition of PEG indicates the highest tannin activity. The increases on gas production in the presence of PEG, were 3.9, 19.5, 91.4, 121.0 and 138.6 % for MAL, MAN, JUR, ARO, and ANG, res-pectively. The treated browses with PEG showed the lowest (P<0.05) tannin bioactivity indicating that this treatment was the most effective approach in deactivation of the tannins in comparison to wood ash or calcium hydroxide treatments.

**Table 1**. Chemical composition (g $kg^{-1}$ DM) and increase in gas production (%) of Brazilian browses

| Plants | Treatments | NFD | ADF | CP | TP[1] | TT[1] | CT[2] | increase |
|---|---|---|---|---|---|---|---|---|
| ANG | control | 524.5 | 298.6 | 153.9 | 93.6 | 88.0 | 13.7 | 138.6 |
| | PEG | 552.3 | 332.9 | 105.8* | 123.4 | 112.7 | 11.3 | 40.9* |
| | ash | 569.9 | 312.4 | 103.8* | 112.5 | 105.8 | 8.5* | 124.9 |
| | $Ca(OH)_2$ | 563.1 | 364.1 | 115.7* | 94.0 | 85.7 | 5.4* | 100.4 |
| | SED | 19.48 | 47.05 | 8.57 | 18.18 | 16.93 | 0.99 | 11.46 |
| ARO | control | 476.6 | 357.8 | 151.6 | 189.5 | 176.5 | 34.3 | 121.0 |
| | PEG | 558.7 | 377.8 | 101.1* | 181.8 | 158.2 | 8.0* | 36.1* |
| | ash | 489.8 | 390.9 | 110.6* | 137.0 | 117.7* | 10.7* | 115.5 |
| | $Ca(OH)_2$ | 558.8 | 388.6 | 111.8* | 125.1* | 112.5* | 7.1* | 120.1 |
| | SED | 23.63 | 44.82 | 4.56 | 13.32 | 12.89 | 3.97 | 15.58 |
| JUR | control | 736.1 | 520.5 | 110.5 | 145.6 | 127.5 | 58.2 | 91.4 |
| | PEG | 544.7* | 416.9* | 140.4 | 127.0 | 107.6 | 7.5* | 5.5* |
| | ash | 610.9* | 474.4 | 171.9* | 87.6* | 71.0* | 12.6* | 33.1* |
| | $Ca(OH)_2$ | 679.4 | 547.3 | 152.2* | 85.7* | 69.9* | 11.0* | 36.1* |
| | SED | 20.38 | 16.32 | 9.63 | 13.16 | 11.59 | 1.73 | 11.18 |
| MAL | control | 742.9 | 383.4 | 109.9 | 9.5 | 6.2 | 0.4 | 3.9 |
| | PEG | 712.1 | 383.2 | 111.0 | 10.4 | 6.6 | 0.5 | 4.8 |
| | ash | 658.1* | 378.2 | 91.9 | 5.7 | 3.5 | 0.1* | 5.4 |
| | $Ca(OH)_2$ | 723.5 | 394.7 | 91.1 | 5.3 | 3.3 | 0.1* | -1.0 |
| | SED | 10.28 | 14.55 | 6.64 | 1.52 | 1.11 | 0.07 | 4.38 |
| MAN | control | 442.8 | 333.0 | 125.5 | 36.2 | 29.9 | 13.5 | 19.5 |
| | PEG | 604.9* | 453.5* | 134.5 | 31.1 | 25.2 | 5.2* | 6.9* |
| | ash | 583.8* | 437.0* | 149.9 | 15.6* | 12.3* | 1.6* | 7.1 |
| | $Ca(OH)_2$ | 639.5* | 465.4* | 144.2 | 11.0* | 8.3* | 1.1* | 21.2 |
| | SED | 20.69 | 18.34 | 8.50 | 2.94 | 2.37 | 1.09 | 3.05 |

[1]TP, total phenols; TT, total tannins as tannic acid equivalents; [2]CT condensed tannins as leucocyanidin equivalents; * means followed by asterisk, within column, are different from control (Dunnett test, P<0.05); SED: standard error of difference between means

**Conclusions** The wood ash and calcium hydroxide treatments were effective only in the case of jurema preta and this may be attributed to the high CT content comparing to the other browses.

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# Effect of monensin and lasalocid on rumen fermentation in sheep

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**Introduction** Ionophores, consisting of monensin and lasalocid were reported to improve rumen fermentation and metabolism of ruminants when delivered in their respective proper dosage (Mass et al., 2001). However, the efficacy of ionophores in the manipulation of rumen fermentation has been shown to vary with diet. Ionophores may also inhibit ruminal amino acid deamination (Yang et al., 2003). The objective of this study was to determine of the effect of monensin and lasalocid on rumen fermentation characteristics.

**Material and methods** 16 sheep (45±6.59 kg) are randomly received one of four diets in a completely randomized design. The composition of diet based on NRC(1985) consisting of 400 g $kg^{-1}$ DM alfalfa , 488 g $kg^{-1}$ DM barley grain, 200 g $kg^{-1}$ DM soybean meal, 589 g $kg^{-1}$ DM, corn grain and 20 g $kg^{-1}$ DM limestone containing predicted metabolizable energy 2.9 M cal $kg^{-1}$ DM and containing crude protein 150 g $kg^{-1}$ DM. The treatments containing different level of ionophores (20ppm, 25ppm, 30ppm and 35 ppm). The period of present study was 21 days for each ionophore. The rumen fluid of each treatment was obtained by stomach tube at 2 hours after feeding. The effect of treatment was determined on rumen pH, ammonia-N, total volatile fatty acid (VFA) (Stuchbury and Sake, 2001), methylene blue reduction time and sedimentation and floatation period. The sedimentation and floatation time was determined using filtration of collected rumen liquor from cheese cloth and then collected in experimental tube, as the small particle precipitated, while large particle was suspended in surface of liquor and the spending time was recorded (Dirksen and Smith, 1987). The methylene blue reduction time was measured using combining of 20 ml rumen liquor and 0.3% methylene blue and recording of reduction time. The data was analyzed using the ANOVA procedure of SAS (SAS Institute, 1987).

**Results** The results are shown in Table 1 & 2. The ammonia–N concentration had significant differences between treatments ($p<0.05$) and the other measured parameters had no significant effects. The less concentration of ammonia-N in 35 ppm of monensin and lasalocid compared to other treatments can be resulted due to reducing the amino acids deamination.

**Table 1** Effect of different level of monensin on rumen metabolites *

| Item | 20 ppm | 25 ppm | 30 ppm | 35 ppm | SEM |
|---|---|---|---|---|---|
| pH | 5.77 | 5.72 | 5.71 | 5.69 | 0.051 |
| Sedimentation and floatation time (sec) | 397.5 | 412.5 | 427.5 | 451.25 | 18.225 |
| Methylene blue reduction time (sec) | 221 | 223.75 | 228 | 230.25 | 3.438 |
| Total VFA mM/L | 124.75 | 125.25 | 126.75 | 127.5 | 3.314 |
| Ammonia-N mg/L | $101^{a}$ | $97.75^{ab}$ | $91.75^{bc}$ | $86.37^{c}$ | 2.851 |

*The means within a row without common letter differ ($p<0.05$)

**Table 2** Effect of different level of lasalocid on rumen metabolites *

| Item | 20 ppm | 25 ppm | 30 ppm | 35 ppm | SEM |
|---|---|---|---|---|---|
| pH | 6.20 | 6.18 | 6.08 | 6.05 | 0.084 |
| Sedimentation and floatation time (sec) | 368.25 | 371.25 | 377.50 | 380.00 | 5.701 |
| Methylene blue reduction time (sec) | 181.00 | 188.75 | 210.00 | 215.50 | 15.378 |
| Total VFA mM/L | 104.87 | 105.25 | 105.62 | 106.37 | 4.008 |
| Ammonia-N mg/L | $89.47^{a}$ | $85.00^{ab}$ | $82.77^{ab}$ | $77.40^{b}$ | 2.681 |

*The means within a row without common letter differ ($P<0.05$)

**Conclusion** The results showed that the ammonia-N was significantly decreased when the diet contained high level of monensin and lasalocid indicated increasing of proportion of intact amino acid in the rumen that could be used in the intestine.

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# Microscopic Studies and Molecular Identification of Ruminal Zygomycetes Fungi in Sheep

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**Introduction** Rumen is an area with high potential for growing of diverse living organisms including anaerobic fungi. The most studies on rumen fungi focused on anaerobic fungi classified as *Chytridiomycetes*, family *Neocallimasticaceas*, while few studies have done on determination of *Zygomycetes* that can be native in the rumen. The most fungi within the subkingdom *Zygomycotina* belong to the class *Zygomycetes*. Fungi in this class possess some distinctive properties that contain rapid growth, nonseptate mycelium, reproduction by sporangiospores (Pitt *et al.,* 1985) and production of some hydrolytic enzymes (Vinogradova *et al*.,2003). The aim of this study was microscopic examination and molecular analysis of nuclear small subunit (SSU; 18S rDNA) of ruminal fungi, *Zygomycete*, and its reproductive manner in sheep.

**Materials and methods** Rumen fluid from a rumen-fistulated sheep, fed a 50:50 concentration:hay ration, was filtered through a layer of muslin and clarified by centrifugation at 10,000 × g for 30 min and used as a source of fungi. Inoculum was cultured anaerobically in reduced medium and incubated at 39 °C for 3 days as described by Joblin (1981). 100 µl of each cultured samples was transferred on sterilized glass slide which was into a sterilized petri dish, and then by potting a cover glass on it and incubated under a $CO_2$ atmosphere at 39 °C for 24 h. Fungal mycelium and their sporangiospores were photographed using a bright- field microscopy. In an attempt to isolate specific fungi, colonies were picked and transferred into sisal broth medium. Genomic DNA was extracted from biomass harvested by centrifugation (2000 Rpm, 10 min) from pure culture grown on sisal broth medium (Fliegerova *et al*., 2004) by Guanidine Thiocyanate-Silica Gel method (Boom *et al*., 1990). Primers NS1 (5´–GTAGTCATATGCTTGTCTC-3´) and NS2 (5´-GGCTGCTGGCACCAGACTTGC-3´) amplified a 550-bp fragment from SSU 18S rDNA (Fliegerova *et al* 2006). In a volume of 20 µl PCR reactions contained: 50 ng of template DNA, 2µl 10-X PCR buffer, 2.5 mM $MgCl_2$, 200 µM each dNTPs, 10 pM of each primer, and 1 U *Taq* DNA polymerase. Thermal conditions for 35 cycles were 95 °C (40 sec), 45 °C (40 sec), and 72 °C (1 min) followed by a final extension at 72 °C (5 min). PCR products were visualized by electrophoresis on 1.3% agarose gel stained with etithium bromide.

**Results** Anaerobically culture of the fungi showed that these fungi are able to grow in rumen condition with sexual reproduction (Figure 1). A 550 bp fragment of the SSU 18S rDNA was amplified successfully from extracted DNA (Figure 2).

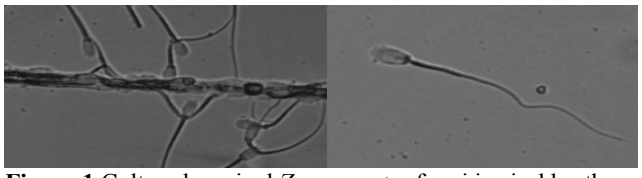

**Figure 1** Cultured ruminal *Zygomycetes* fungi in sisal broth medium.

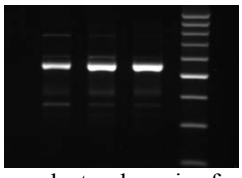

**Figure 2** Agarose electrophoresis of a 550 bp fragment of the SSU 18S rDNA.

**Conclusions** Result of this study demonstrated that there are some *Zygomeycetes* fungi that can be grown anaerobically in the rumen of sheep and since they can be able to produce hydrolytic enzymes, such as cellulose, amylase and protease, therefore further studies about these organisms in the rumen is useful, and PCR method can be used for exact identification of these fungi.

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# The chemical composition and *in vitro* digestibility of pistachio by-product

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**Introduction** Pistachio (*pistachio vera*) is from Anacardiaceae family. According to the FAO (2003) report, Iran is the largest pistachio producer in the world (more than 310,000 tons). The most three important exported pistachio nuts of Iran are Ohadi, Akbari and Kaleghuchi (Mohammadi, 2005). About 150,000 tons in DM of pistachio by-product (PB) is produced from dehulling process in Iran, annually. This by-product is mainly consisted of pistachio hulls (PH), and then peduncles, leaves and a little amount of mesocarp and kernels. In this experiment the chemical composition, Total Phenolic Content (TPC) and tannin amount of PB and PH of Ohadi, Kaleghuchi and white varieties were determined; also, *In Vitro* Dry Matter and Organic Matter Digestibility (IVDMD and IVOMD) were measured.

**Materials and methods** Ten samples of each PB and PH variety were collected from Toosdasht factory in Torbat in Iran and their chemical compositions determined in confirm with AOAC (1990). Total Phenolic Content (TPC) and tannin were determined by using Folin-Ciocalteu reagent (Makkar and Singh, 1992). To determine IVDMD, ingredients were grounded through a 1mm mill screen and incubated in double stages (incubation in a ruminal fluid (from fistulated sheep) and artificial saliva mixture medium for 48h and expose to the HCl and Pepsin solutions for 48h), according to the Tilley and Terry (1963). There were 4 and 3 replicates for PBs and PHs, respectively. After incubation, the incubation medium were filtered from Watman filter sheets (No. 42) and dried in the oven. Afterwards the ash quantity of dried filtered matter was detected to determine the IVOMD. The experimental data were analyzed as a complete randomized design by using the procedure of SAS (2002).

**Results** There were some differences between chemical compositions of different pistachio by-products and their hulls. As be seen in table 1 crud protein content of Kaleghuchi pistachio by-product was lower than others and the White variety had the lowest tannin level among them. According to the table 2, the IVDMD and IVOMD of different PBs were significantly different ($P<0.01$), but for PHs were not differed significantly ($P>0.05$). The IVDMD and IVOMD of Kaleghuchi pistachio by-product were higher than others, although it had more tannin, ash and NDF and lower CP than Ohadi and White cultivars. In other hand there were not significant differences between *in vitro* digestibilities of different hulls.

**Table 1** Chemical composition of different pistachio by-products and hulls

| Item | Ash | EE | CP | NDF | ADF | TPC | Tannin |
|---|---|---|---|---|---|---|---|
| | | Total by-product | | | | | |
| Ohadi | 9.1 | 8.7 | 14.2 | 25 | 20.5 | 8.6 | 4.1 |
| Kaleghuchi | 13 | 7.8 | 9.4 | 24 | ND[1] | 9.5 | 4.6 |
| White | 12 | 7 | 16.6 | 27 | ND | 7.6 | 3.4 |
| | | Hulls | | | | | |
| Ohadi | 12 | 5.7 | 16.6 | 25 | 20 | 9.6 | 4.5 |
| Kaleghuchi | 15 | 7.8 | 9.2 | 22 | ND | 9.1 | 4.4 |
| White | 14 | 5.7 | 17.7 | 24 | ND | 7.5 | 3.2 |

[1]not detected

**Table 2** *In vitro* digestibility of different pistachio by-product and hulls

| (%) | Ohadi | Kaleghuchi | White | SE | P |
|---|---|---|---|---|---|
| Total by-product | | | | | |
| IVDMD | 56.0[c] | 63.7[a] | 61.0[b] | 0.14 | ** |
| IVOMD | 58.0[c] | 68.4[a] | 64.1[b] | 0.12 | ** |
| Hulls | | | | | |
| IVDMD | 63.5 | 67.0 | 66.0 | 0.29 | NS[1] |
| IVOMD | 67.4 | 71.9 | 70.9 | 0.24 | NS |

** $P<0.01$, [1]$P>0.05$

**Conclusion** The *in vitro* digestibility of the pistachio hulls was higher than the total pistachio by-products. The IVDMD and IVOMD of Kaleghuchi by-product were significantly higher than other varieties. The differences between *in vitro* digestibility of different pistachio by-products seems to be dependent on other factors in addition to their determined chemical composition, such as the portion of each components (hulls, peduncles, leaves, mesocarps and kernels) in the pistachio by-products.

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# The chemical composition and ruminal disappearance of pistachio by-product

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**Introduction** Iran is the greatest pistachio producer in the world. A large quantity of pistachio by-product (at least 150,000 tons in DM) is produced from pistachio dehulling process in Iran, annually. The results of some experiments show this by-product can be used as a feedstuff for ruminants (Shakeri and Fazaeli, 2005; VAhmani *et. al.,* 2005). However, more information about physical and chemical compositions of this by-product is needed. Pistachio by-product is mainly consists of epicarp, peduncle, leaf and a little amount of mesocarp and kernel. The aim of this study was to determine chemical composition and *in situ* dry matter disappearance of each part of pistachio by-product (Ohadi variety) after 0, 12 and 24 h incubation in the rumen.

**Materials and methods** Ten samples of Ohadi pistachio by-product were collected from Toosdasht factory in Torbat in Iran and separated their constituents, annually; then the chemical composition of them were determined in confirm with AOAC (1990). Total Phenolic Components (TPC) and tannin content of them were detected according to the Folin-Ciocalteu method (Makkar and Singh, 1992). *In situ* ruminal disappearances of different parts of by-product were measured by using ruminal nylon bags (12×17 cm). Ingredients were 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 steers. Steers were fed at maintenance level. The bags were removed at 12 and 24 hours 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.

**Results** Table 1 shows portion and chemical compositions of each pistachio by-product constituents. The pistachio epicarp (hulls) was the main part of this by-product. The kernel had the highest amount of fat and protein than the other parts, but it had the lowest amount of portion. The leaves had highest TPC and tannin quantity, but this part was disappeared in the rumen more than the peduncles. About 77% of the kernel was disappeared in the water ($t_0$), although just 5% of mesocarp was disappeared at this time. The hulls were disappeared more than the peduncles and leaves after 24 h incubation in the rumen. More than 50% of the pistachio by-product was washed out with running tap water, but only 20% of it was disappeared up to 24 h incubation in the rumen (table 2).

**Table 1** Chemical composition of different parts of pistachio by-product (percent in DM)

| Item | Portion (%) | Ash | EE | CP | NDF | NFC | ADF | TPC | Tannin |
|---|---|---|---|---|---|---|---|---|---|
| Epicarp (hull) | 53.5 | 12.7 | 5.7 | 16.6 | 25 | 40 | 20.0 | 9.6 | 4.5 |
| Peduncle | 27.7 | 5.6 | 7.1 | 12.1 | 39 | 36.2 | ND[1] | 10.0 | 4.8 |
| Leaf | 9.5 | 9.2 | 3.8 | 12.4 | 31 | 43.6 | ND | 13.9 | 6.9 |
| Mesocarp | 5.3 | 0.9 | 0.3 | 1.6 | 91 | 6.2 | ND | 1.5 | 0.5 |
| Kernel | 4.0 | 2.8 | 48.0 | 24.5 | ND | 24.7 | ND | 1.2 | 0.3 |
| To. By-product | 100 | 9.1 | 8.7 | 14.2 | 25.5 | 42.5 | 20.5 | 8.6 | 4.1 |

[1] Not Detected

**Table 2** Dry matter disappearance ratio (g/g) after ruminal incubation of pistachio by-product constituents

| | Incubation time (h) | | | |
|---|---|---|---|---|
| Item | 0 | 12 | 24 | n |
| Epicarp | 0.519±0.00 | 0.699±0.09 | 0.773±0.04 | 3 |
| Peduncle | 0.372±0.01 | 0.534±0.10 | 0.598±0.05 | 3 |
| Leaf | 0.360±0.00 | 0.626±0.21 | 0.659±0.12 | 3 |
| Mesocarp | 0.050±0.00 | 0.103±0.02 | 0.221±0.04 | 3 |
| Kernel | 0.769±0.00 | 0.864±0.09 | 0.921±0.07 | 3 |
| To. By-product | 0.506±0.04 | 0.653±0.09 | 0.698±0.05 | 3 |
| SE | 0.15 | 0.26 | 0.23 | |

**Conclusion** Although soluble part of this pistachio by-product was relatively high, but its degradability in the rumen was fairly low. The Kernel and mesocarp disappeared in the rumen in the highest and lowest amount, respectively; but the Peduncles was the less digestible part of the feed regarding to its portion in this by-product (27.7 *vs.* 5.3% of Peduncle and Mesocarp, respectively) and it seems that omitting of this part, can improve digestibility and palatability of this by-product for the ruminants.

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# Chemical composition of rice bran and fatty acid profile of the oil fraction

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**Introduction** Rice bran is the most important by-product obtained during the rice milling process. The chemical composition of rice bran varies widely that is related to the milling system and management (Ravindran and Blair, 1991). Therefore, the terminology concerning rice bran is not consistent due to the lack of well-based definitions. Rice bran contains a considerable amount of crude protein and fat which, in many countries, is now being extracted to obtain a high quality of oil. The high fat in rice bran may create rancidity problems during storage in tropical climates. The bran also contains a lipolytic enzyme that becomes active when it is separated from the rice and rapidly increase the free fatty acid content but, the storage life of solvent-extracted bran is considerably longer than the un-extracted one (Warren and Farrel, 1990). In Iran, annually about 200000 tons of this by-product is available which is used as an animal feed without any extraction process. This study was conducted to study the chemical composition and fatty acids profile of the oil fraction in rice bran during the different seasons.

**Material and methods** A stratified randomised sampling method was carried out, in which, 60 rice milling unit were selected, out of 200 processing plant, from 20 regions of Gilan Province in Iran. Each unit was visited three times during the year (autumn, winter and spring) and samples were taken from stored rice bran. Chemical compositions were determined according to the standard methods and fatty acids component measured, using gas chromatography technique (A.O.A.C. 1990). Data were statistically analysed according to the complete randomised design and tested for significance, between seasons, using Duncan multiple range test.

**Results** Table 1 shows the chemical composition of rice bran in different seasons. Except for the nitrogen free extract (NFE), there were no significant differences (P>0.05) among the samples of different seasons for chemical compositions and gross energy (GE) content. Comparing to the standard tables of the NRC (1989), the crude protein (CP) of the Iranian rice bran was lower and the crude fibre (CF), neutral detergent fibre (NDF) and acid detergent fibre (ADF) were higher. The concentration of fatty acids including: Luric (Lu), Myrestic (My), Palmitic (Pl), Stearic (St), Oleic (Ole), Linoleic (Li), Linolenic (Lin) and Arachidonic (Arch) are presented in Table 2. The main fatty acids components were Ole, Li and Pl respectively. The concentration of Ole and Li acids were significantly (P<0.05) different between the seasons but, Pl acid content was not variable. During the spring, Ole was significantly (P<0.05) lower than the other seasons, but the concentration of Li was the highest (P<0.05) during the spring. The correlation coefficient between the chemical compositions was as follow: ADF with NDF (r = 0.9); CF with ADF (r =0.9); CF with NDF (r =0.86) and CF with CP (r =0.85).

**Table 1** Chemical composition (based on g/kg DM) and GE (based on MJ/kg DM)

| Season | DM | CP | EE | Ash | CF | NFE | NDF | ADF | ***GE*** |
|---|---|---|---|---|---|---|---|---|---|
| Spring | 908±14 | 89.5±16 | 69±15 | 124±28 | 256±55 | 369$^{a}$ ±59 | 466±58 | 311± 62 | 17.5±1.5 |
| Autumn | 929±11 | 80.1±16 | 76±15 | 141±17 | 281±46 | 350$^{a}$ ±38 | 483±39 | 323± 42 | 16.8±0.7 |
| Winter | 920±18 | 81.6±27 | 95±33 | 144±34 | 287±81 | 313$^{b}$ ±64 | 469±86 | 316± 81 | 17.3±1.14 |
| significance | ns | ns | ns | ns | ns | * | ns | ns | ns |

ns = non significant, *= (P <0.05)

**Table 2** Fatty acids profile, based on the gram per 100 gram of total fatty acids at different seasons

| Season | Lu (12:0) | My (C14:0) | Pl (C16:0) | St (C18:0) | Ole (C18:1) | Li (C18:2) | Lin (18:3) | Arch(20:4) |
|---|---|---|---|---|---|---|---|---|
| Spring | 0.06$^{a}$ ±0.02 | 0.35±0.07 | 18.95±1.10 | 0.92$^{b}$ ±0.12 | 41.6$^{b}$±4.46 | 35.9$^{a}$±3.3 | 1.2±0.2 | 0.03$^{b}$±0.03 |
| Autumn | 0.03$^{b}$±0.02 | 0.25±0.06 | 18.86±1.70 | 1.27$^{b}$±0.31 | 45.0$^{a}$±2.37 | 33.1$^{b}$±2.2 | 1.3±0.3 | 0.70$^{a}$±0.40 |
| Winter | 0.05$^{a}$±0.05 | 0.40 ±0.07 | 18.33±1.20 | 1.87$^{a}$±0.17 | 44.0$^{a}$±1.7 | 32.0$^{b}$±1.7 | 1.3±0.3 | 0.70$^{a}$±0.20 |
| significance | * | ns | ns | * | * | ** | ns | ** |

ns = non significant, *= (P <0.05), ** = (P<0.1)

**Conclusion** The Iranian rice bran with high CF, NDF, and ADF and low CP could not be considered as standard rice bran, it may be defined as rice milling by-product, which is a mixture of outermost layers of the rice grain (pericarp, tegment and aleurone layers), the embryo, broken endosperm and varying levels of rice hulls. Concentration of the major fatty acids during the different seasons could be similar to the standard that explained by Gustone and Wolff, (1996).

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# Nutrient composition of wheat grain and wheat bran in East Azerbaijan province of Iran

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**Introduction** In Iran, East Azerbaijan province is one of the most important farm animal production area and feedstuffs such as wheat grain (WG) and wheat bran (WB) together with alfalfa hay, barley grain, and wheat straw are the most common ingredients of animal rations. In recent years, production of WG and its main by-product, WB, have considerably been increased. Organic matter and mineral contents of WG in a given area can be influenced markedly by climate, soil and fertilizer treatments, growth stage and agronomic factors. Nutrient composition of WB also depends on variety of WG and mill processing conditions. Therefore, it is necessary to determine the nutrient composition of WG and WB to use in formulating balanced rations. The objective of present study was to determine nutrient composition of WG and WB in East Azerbaijan.

**Materials and methods** Using Probability Stratified Random Sampling, East Azerbaijan province was classified into 30 agricultural zones. Totally, 124 villages were selected for taking samples randomly. WG was subjected to sampling according to scientific methods from stack and depots of 3 to 5 farmers and fifteen samples of WB were taken from 15 mill factories in the province. All samples were subjected to preliminary drying and grounding. Equal amounts of WG samples belonging to villages of a given zone, were mixed and then a new composite sample resulted. In this way, 27 samples were resulted for WG. All samples were then grounded through a 1 mm screen prior to chemical analysis. Dry matter (DM), Crude Protein (CP), Ether Extract (EE) and ash and some minerals (Ca, P, K, Mg, Na) contents of feeds were measured according to AOAC (1990). Acid Detergent Fibre (ADF) and Neutral Detergent Fibre (NDF) according to Goering and Van Soest (1970) procedure. The data was subjected to statistical analysis by t-test procedure (with Ho= m option) of SAS.

**Results** In most cases, the mean values of chemical composition and mineral contents, except ash and P, in WG were significantly ($P<0.01$) different from those reported by NRC (2001). DM and CP contents of WG were significantly higher and lower than the corresponding values in NRC (2001). CP content of WG was as much as 12.4 g/kg lower than that in NRC (2001). In the case of EE, significant differences were not found between WG and NRC (2001). NDF and ADF contents of WG showed significant differences ($P<0.01$) with those in NRC (2001). NDF was as much as 41.5 g/kg more than that reported by NRC (2001). NFC value was 667.4 g/kg DM in WG. Except P, mean values of Ca, Mg, K and Na contents in WG showed significant differences ($P<0.01$) with those in NRC(2001). Except CP and EE, the values of DM, NDF, ADF and Ash in WB were significantly different ($P<0.01$) from those in NRC (2001). DM and NDF of WB were more and ADF was too lower than NRC (2001), respectively. The mean values of K and Mg in WB were significantly higher than those in NRC (2001). Ca, P and Na contents in WB were not significantly different from those in NRC (2001).

**Table 1** Mean (g/kg DM) and standard deviation (in parentheses) data of chemical composition and some macro elements of WG and WB in East Azerbaijan and their comparisons with the corresponding data with NRC (2001).

| Item | WG (n=27) | NRC (2001) | Probability | WB (n=15) | NRC | Probability |
|---|---|---|---|---|---|---|
| DM | 909.3 (3.2) | 894 | ** | 915 (9.3) | 891 | ** |
| CP | 129.6 (10.7) | 142 | ** | 178.9(11.9) | 173 | NS |
| EE | 21.1 (0.57) | 23 | ** | 43.7 (3.48) | 43 | NS |
| NDF | 175.5 (21.1) | 134 | ** | 457.2(36.7) | 425 | ** |
| ADF | 36.1(6.1) | 44 | ** | 147.2 (14.8) | 155 | ** |
| NFC | 667.4 (54.1) | - | | 136.4(49.5) | - | |
| Ash | 19.4 (4.2) | 20 | NS | 59 (10.74) | 63 | ** |
| Ca | 3.3 (0.67) | 0.5 | ** | 1.46 (0.35) | 1.3 | NS |
| P | 4.9 (1.8) | 4.3 | NS | 11.5 (3.1) | 11.8 | NS |
| K | 5.8 (0.64) | 5 | ** | 15.8 (1.75) | 13.22 | ** |
| Mg | 4.8 (2.2) | 1.5 | ** | 10.4 (2.1) | 5.3 | ** |
| Na | 0.21 (0.06) | 0.1 | ** | 0.33 (0.11) | 0.4 | NS |

** $P<0.01$, NS=non significant

**Conclusion** The results showed that the nutrient composition of WG, except for P, is considerably different from those which have been reported by NRC, so the presented data here on WG are recommend in applied animal feeding instead of NRC data, which is more common in animal production system in Azerbaijan. In the case of WB, it is recommend that values reported here for DM, NDF, ADF, NFC, Mg and K be used when formulating rations.

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# 'Grape marc' as a source of tannins to reduce proteolysis during ensilage

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**Introduction** A reduction of the overall nitrogen (N) loss to the environment from intensive ruminant production systems remains a key policy and research objective. Such an outcome is essential for the development of sustainable ruminant farming systems that minimise their environmental footprints. Plant and microbial enzyme-mediated proteolysis during ensilage results in the extensively hydrolysed N fraction in grass silage (Givens and Rulquin, 2004). It is generally accepted that the highly soluble N fraction of grass silage is poorly utilised by ruminants, reflecting the low efficiency of capture of silage N in the rumen. An improvement in dietary N efficiency by ruminants is therefore a key concern. Plant tannins have in common their capacity to bind proteins and an improvement in N utilisation by ruminants has been reported for a number of tanniniferous feeds (Mueller-Harvey, 2006). Therefore, the aim of the present experiment was to study a by-product from the wine industry, so-called 'grape marc', as a potential source of tannins to reduce proteolysis during ensilage.

**Materials and methods** Grass silage treatments were prepared from 1st cut (May 2005) perennial ryegrass (dry matter (DM), 218.8 g/kg; N, 21.8 g/kg DM). The herbage was mown with a mower conditioner, wilted (~24 h) and then picked up with a precision chop forage harvester and stored frozen (-20°C) prior to use. Fresh grape marc, the by-product remaining following the pressing of grapes (Reichensteiner variety) for wine manufacture, comprising mainly of grape skins and seeds, was stored frozen (-20°C) immediately following collection. A sample of the grape marc was freeze-dried and milled (1 mm screen; residual DM, 909.5 g/kg; N, 10.9 g/kg DM) prior to ensilage. A randomised complete block design was employed with seven silage treatments; control (no grape marc) and increasing amounts of grape marc (target rates: 60, 120, 180, 240, 300 and 360 g DM/kg grass DM) in three blocks. The grape marc was applied to the grass immediately prior to ensilage in laboratory-scale silos (~230 g grass/silo) and kept in a temperature controlled environment (~20°C) for ~90 d. Sub-samples of the fresh silages were analysed for DM (100°C for 18 h), ash, total N (TN; Kjeldahl method) and ammonia-N ($NH_3$-N) contents and pH. The data were analysed using ANOVA with effects for treatment and block. Analysis of contrasts (e.g. control with mean of grape marc-treatments) and the linear treatment effect were also completed.

**Results** A difference between the actual DM content of the grass pre-ensilage and the DM value used in the initial calculations (218.8 and 266 g/kg respectively) resulted in the grape marc being added at rates of ~73, 146, 219, 292, 365 and 438 g DM/kg grass DM (equivalent to ~68, 127, 180, 226, 267 and 304 g/kg DM). The composition of the grape marc-treated silages is presented in Table 1. Addition of freeze-dried grape marc gave a significant linear ($P<0.001$) increase in DM ($P<0.001$) and decrease in N ($P<0.01$) content of the silage treatments. The $NH_3$-N content (range, 3.49 to 2.35 g/kg DM) and the proportion of TN as $NH_3$-N (range 14.0 to 10.8 g/100 g) were significantly ($P<0.001$) lower in the grape marc-treated silages.

**Table 1** Chemical composition of grape marc-treated silages (as g/kg DM unless stated otherwise)

| | Level of added grape marc (g DM/kg grass DM) | | | | | | | Contrast[1] | Contrast[2] | Linear |
|---|---|---|---|---|---|---|---|---|---|---|
| Parameter | 0 | 73 | 146 | 219 | 292 | 365 | 438 | s.e.d. | s.e.d. | effect |
| DM (g/kg) | 196.0 | 214.8 | 216.2 | 222.5 | 233.8 | 239.2 | 246.6 | 3.84 | 2.94 | *** |
| pH | 3.98 | 4.03 | 3.84 | 3.88 | 3.93 | 3.85 | 3.84 | 0.053 | 0.040 | ** |
| TN | 24.9 | 23.5 | 23.0 | 23.3 | 22.4 | 22.7 | 21.7 | 0.523 | 0.400 | *** |
| $NH_3$-N | 3.49 | 3.08 | 2.92 | 2.87 | 2.59 | 2.61 | 2.35 | 0.097 | 0.074 | *** |
| $NH_3$-N/TN[3] | 14.0 | 13.1 | 12.7 | 12.3 | 11.6 | 11.5 | 10.8 | 0.431 | 0.329 | *** |
| $NH_3$-N/N[4] | 0.142 | 0.139 | 0.135 | 0.139 | 0.133 | 0.139 | 0.131 | 0.005 | 0.003 | NS |
| Ash | 115.4 | 114.3 | 109.7 | 109.0 | 112.3 | 106.9 | 105.5 | 2.21 | 1.69 | *** |

Contrast[1], comparison of treatment means; contrast[2], comparison of control with mean of grape marc treatments; s.e.d., standard error of difference; ***, $P<0.001$; **, $P<0.01$; NS, non-significant; [3], proportion of TN as $NH_3$-N (g/100 g); [4], weight of silage $NH_3$-N/weight grass N ensiled (g/g).

**Conclusions** Ensilage of grass with freeze-dried grape marc resulted in a significant reduction in $NH_3$-N content of the silages, suggesting a reduction in proteolysis (amino acid deamination) of the grass N fraction during ensilage. This result is likely to reflect the increasing proportion of grape marc within the overall treatment DM (range 0 to 304 g/kg DM) since there was no significant effect ($P=0.283$) of treatment on $NH_3$-N when the results were expressed as the weight of silage $NH_3$-N/weight grass N ensiled (g/g) in each silo (e.g. 0.142 and 0.136 g $NH_3$-N /g grass N ensiled for control and mean of grape marc treatments respectively). Further work is required to determine the effect of grape marc and other tannin-containing agro-industrial by-products on the various silage N fractions and *in vivo* utilisation of forage N.

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Mueller-Harvey, I. 2006. Review: Unravelling the conundrum of tannins in animal nutrition and health. *Journal of the Science of Food and Agriculture*, **86**:2010-2037.

# Non-toxic plant tannins as silage additives: effect on proteolysis during ensilage

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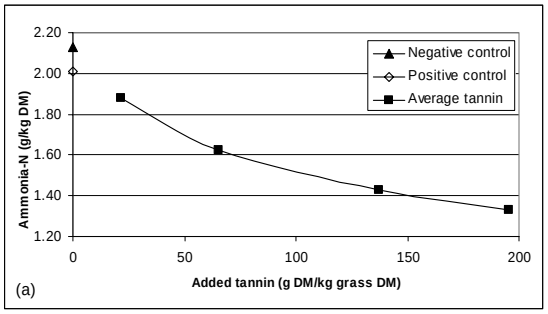
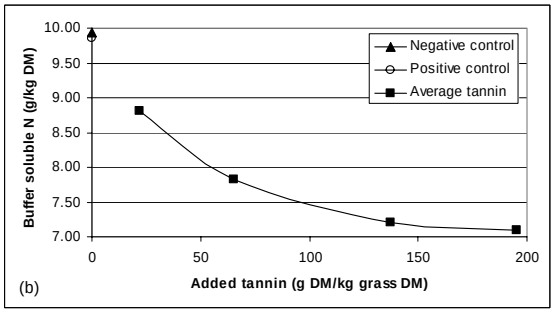
**Introduction** Sustainable ruminant livestock systems must promote an efficient use of resources for food production and minimise their environmental impact. Intensive dairy cow production, based on grass silage, is a major contributor to nitrogen (N) loss to the environment, estimated as 18.2 t N/t milk for grass silage-based diets (Delaby et al., 1995). It is widely accepted that this low efficiency (milk N/dietary N) value reflects the low efficiency of capture in the rumen of the extensively degraded N fraction in grass silage (Givens and Rulquin, 2004). A common feature of plant tannins is their capacity to bind proteins and an improvement in N utilisation by ruminants has been reported for a number of tanniniferous feeds (Mueller-Harvey, 2006). Therefore, the aim of the present experiment was to study the potential of various commercial non-toxic tannin products to reduce proteolysis during ensilage.

**Materials and methods** Grass silage treatments were prepared from 1st cut (May 2005; dry matter (DM) 245.2 g/kg; 21.8 g N/kg DM) perennial ryegrass. The herbage was mown with a mower conditioner, wilted (~24 h) and then picked up with a precision chop forage harvester and stored frozen (-20°C) prior to use. A randomised complete block design was employed with 46 treatments: negative control (no added tannins), positive control (formic acid-treated; equivalent to 3 l/t fresh grass) and tannin-treated silages involving 11 tannins (including, chestnut, mimosa and tara tannins), added in increasing amounts (target rates: 20, 60, 120 and 180 g/kg grass DM) in three blocks. The tannin powders were sieved onto the grass and then incorporated using 11.5 ml distilled water/230 g fresh grass. All treatments were ensiled in laboratory-scale silos (~230 g grass/silo) and kept at ~20°C for 90 d. Sub-samples of the silages were analysed for DM (100°C for 18 h), total N (Kjeldahl method) and ammonia-N ($NH_3$-N) contents. Dried silages (100°C for 8 h) were used to determine buffer soluble N (BSN) content. The data were analysed using ANOVA with effects for treatment and block.

**Results** A small difference between the actual DM content of the grass pre-ensilage and the DM value used in the initial calculations (245.2 and 266 g/kg respectively) resulted in the tannins being added at rates of 21.7, 65.1, 130.2 and 195.3 g DM/kg grass DM. There was a significant ($P<0.001$) effect of tannin and rate of tannin addition on the composition of the tannin-treated silages. A significant tannin x rate of tannin interaction was recorded for DM ($P<0.05$), $NH_3$-N ($P<0.001$) and BSN ($P<0.01$) content. The effect of tannin (mean across 11 tannins) and rate of tannin addition on $NH_3$-N and BSN content is summarised in Figure 1(a) and (b) respectively.

**Figure 1** Effect of tannin (mean across 11 tannins) and rate of tannin addition on (a) $NH_3$-N and (b) BSN content of tannin-treated silages (as g/kg DM)




| Effect on $NH_3$-N | df | F | P-value |
|---|---|---|---|
| Tannin | 10 | 13.3 | <0.001 |
| Rate | 3 | 232.6 | <0.001 |
| Tannin x rate | 30 | 2.33 | <0.01 |
| s.e.d. = 0.075 | | | |

| Effect on BSN | df | F | P-value |
|---|---|---|---|
| Tannin | 10 | 27.6 | <0.001 |
| Rate | 3 | 54.1 | <0.001 |
| Tannin x rate | 30 | 2.70 | <0.001 |
| s.e.d. = 0.499 | | | |

df, degrees of freedom; s.e.d., standard error of difference of any two treatment means.

**Conclusions** The $NH_3$-N and BSN components of the silage N fraction were reduced by treatment with plant tannins both in relation to the controls and with increasing level of added tannin. A small difference in the BSN content of the control treatments was observed when expressed as g/100 g total N. Further work is required to determine the potential benefits of plant tannins on other silage N fractions and *in vivo* utilisation of forage N.

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Mueller-Harvey, I. 2006. Review: Unravelling the conundrum of tannins in animal nutrition and health. *Journal of Science Food and Agriculture*. **86**:2010-2037.

# Chemical characteristics of Lucerne silage treated by urea and sulphuric acid

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**Introduction** Although Lucerne hay is common using method of Lucerne forage but weather condition always is not suitable to provide it. Besides Lucerne forage due to high buffering capacity because of the tubular and hollow stem structure, low soluble sugar content, high moisture and high protein content is the most difficult to ensile. Therefore, response to silage additives (such as urea and sulphuric acid) or preservatives may be great with legume forage. Experimental evidence indicates that there is main different about chemical composition of Lucerne silage before and after ensiling**.** This study was conducted to compare of Chemical composition of Lucerne silages treated without or by urea and sulphuric acid.

**Materials and methods** Second growth Lucerne (about 30% DM) was harvested and chopped to a length of 8 to10 cm, then ensiled with urea and /or sulphuric acid. Silages were provided in laboratory silos (2 repeats in every treatment) lined with two layers of plastic, after air exclusion. The experimental design was a randomized complete design with a 3*3 factorial arrangement of treatments. Silages were Lucerne silage, Lucerne silage + 0.6% sulphuric acid, Lucerne silage + 1.2% sulphuric acid, Lucerne silage + 0.5% urea, Lucerne silage + 0.5% urea + 0.6%sulphuric acid, Lucerne silage + 0.5% urea + 1.2% sulphuric acid, Lucerne silage + 1% urea, Lucerne silage + 1% urea + 0.6 sulphuric acid and Lucerne silage + 1% urea + 1.2% sulphuric acid. Main effects were urea (0.0, 0.5 and 1% of DM) and sulphuric acid (0.0, 0.6 and 1.2 % of DM). The chemical compositions of the silages were determined after 40 days of ensiling. The standard procedures were used to determine the chemical compositions of samples (AOAC, 1992). PH and N-$NH_3$ were determined in silage extraction. Crud Protein (CP), organic mater (OM) and non protein nitrogen (NPN) were determined in dry sample of silages. The general linear models (GLM) procedure of SAS (3) was used for analysis of dependent variables. Urea and sulphuric acid levels interaction were included in the model. When the interaction was significant ($P$ □0.05), *t*-tests were used to identify between-group differences. Statistical significance was determined at P<0.05.

**Results** Chemical composition of the silages treated by urea and sulphuric acid are shown in Table 1. The addition of urea to the silage increased significantly CP, NPN, N-$NH_3$ content and PH (P<0.05). Using sulphuric acid as an additive of silage caused to reduce PH and N-NH3, NPN content of the silage (P<0.05). However, DM, OM and NPN/N were not significantly influence by treatments (P<0.05).

**Table 1** Chemical composition of Lucerne hay or the silages treated by urea and sulphuric acid

| Item | Treatments | | | | | | | | | | Effects | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $U_0$ | | | $U_{0.5}$ | | | $U_1$ | | | | | | |
| | $S_0$ | $S_{0.6}$ | $S_{1.2}$ | $S_0$ | $S_{0.6}$ | $S_{1.2}$ | $S_0$ | $S_{0.6}$ | $S_{1.2}$ | SEM[1] | U | S | U*S |
| PH | 4.4 | 4.1 | 3.8 | 4.8 | 4.3 | 4.2 | 4.7 | 4.3 | 4.2 | 0.1 | * | * | * |
| DM (g/kg$^{-1}$) | 243 | 327 | 325 | 307 | 336 | 327 | 347 | 224 | 332 | 58.3 | ns | ns | ns |
| OM (g/kg$^{-1}$) | 936 | 929 | 921 | 925 | 922 | 918 | 930 | 921 | 915 | 5.3 | ns | ns | ns |
| CP (g/kg$^{-1}$) | 181 | 174 | 181 | 183 | 189 | 197 | 185 | 213 | 214 | 8.2 | * | ns | ns |
| NPN (g/kg$^{-1}$) | 19 | 13 | 15 | 13 | 15 | 18 | 15 | 19 | 18 | 2.2 | * | * | * |
| NPN/N (g/g) | 0.68 | 0.47 | 0.52 | 0.46 | 0.51 | 0.58 | 0.49 | 0.57 | 0.54 | 0.06 | ns | ns | ns |
| N-$NH_3$ (mg dl$^{-1}$) | 12.2 | 10.6 | 8.7 | 13 | 10.1 | 10.7 | 14.9 | 12.8 | 9.8 | 1.7 | * | * | ns |

[1]Standard error of mean.
*: P< 0.05.
NS: non significant.

**Conclusion** The results of the current study indicated that the urea as an additive for Lucerne silage, increase CP of silages. While the NPN content of the silages treated with urea was lower compared with LS. Sulphuric acid can also be used as a useful additive in Lucerne silage. Sulphuric acid decreases PH of the silages very quickly. Therefore, sulphuric acid caused to decrease the CP hydrolysis to NPN during fermentation. Besides, sulphuric acid as an additive can increase the DM and protein digestibility of the silages (Delavar, 2003). So it has concluded that both urea and sulphuric acid can be as a useful preservative in Lucerne silage.

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# Changes in chemical composition and *in vitro* DM digestibility of urea and molasses treated whole crop canola silage

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**Introduction** Forage quality is a key factor in dairy cattle nutrition. High moisture forages are so susceptible to loss their nutrients during ensiling. Canola is one of the oilseed plants that belong to Brassica species. Canola forage has high level of moisture in its tissues and must be wilted to 60 - 65% moisture before ensiling. On the other hand, molasses and urea as two silage additives can be effective in improvement and preservation of canola forage quality. Molasses is commonly used to provide readily available energy for lactic acid fermentation. Addition of molasses can increase dry matter content of silage that related to relatively high dry matter content of molasses (Baytok and Aksu, 2005). Meanwhile, silage protein content can be increased and proteolysis decreased with the addition of urea. Application of urea to harvested forage before ensiling can restrict the fermentation processes and release ammonia to potentially enhance nutritive value of the ensiled crop and reduce deterioration during storage. The main objective of this study was to evaluate effects of different levels of urea and molasses on chemical composition and *in vitro* DM digestibility of whole crop canola silage.

**Material and methods.** Whole crop canola was ensiled in plastic containers using additives in a 3×3 factorial arrangement in a completely randomized design with three replicates. Each plastic container had about 1 Kg capacity. The additive levels were 0, 4, and 8% of canola forage DM for molasses and 0, 0.5 and 1 percent of canola forage DM for urea. In order to complete fermentation process, all silages were kept in a cool and dry place for 45 days. After that, silages were opened and analyzed for DM, OM, CP, NDF and ADF contents. Dry matter content of all silage samples was determined by oven drying at 60.c for 48h. DM digestibilities of all silages were estimated by Terry and Tilly (1963) method. Data were analyzed using the general linear model procedure of SAS (2000).

**Results**: Chemical composition and DM digestibility of canola silages are shown in Table 1. Urea had significant effect ($p< 0.05$) on DM, CP, pH and $NH_3$-N. Meanwhile, the EE, NDF, ADF and DM digestibility were similar among groups. Effect of molasses on DM, NDF, ADF and DM digestibility were significant ($p< 0.05$) but CP, EE, pH and $NH_3$-H were similar among groups. The interaction effects between urea and molasses were significant for canola silage DM, EE, NDF, ADF and DM digestibility percentage.

**Table 1** The mean chemical compositions and DM *in vitro* digestibility of canola forage silage treated with different levels of urea and molasses

| Urea | 0[a] | | | 0.5 | | | 1 | | | | Effects | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Molasses | 0 | 4 | 8 | 0 | 4 | 8 | 0 | 4 | 8 | SE | U | M | U×M |
| DM[b] | 190.2 | 197.9 | 202.1 | 186.2 | 188.2 | 208.3 | 186.3 | 199.3 | 199.4 | 0.45 | * | * | * |
| CP | 156.8 | 156.4 | 158.4 | 163.9 | 160.6 | 166.7 | 166.7 | 162.2 | 163.4 | 0.25 | * | ns | ns |
| EE | 60.0 | 43.3 | 40.0 | 43.3 | 33.3 | 36.6 | 43.3 | 36.6 | 43.3 | 0.59 | ns | ns | * |
| NDF | 403.3 | 356.6 | 330.0 | 370.0 | 396.6 | 356.6 | 383.3 | 366.6 | 386.6 | 0.98 | ns | * | * |
| ADF | 323.3 | 313.3 | 300.0 | 320.0 | 316.6 | 313.3 | 320.0 | 346.6 | 310.0 | 0.76 | ns | * | * |
| pH | 4.78 | 4.7 | 4.71 | 4.91 | 4.97 | 4.84 | 5.05 | 5.23 | 5.07 | 0.11 | * | ns | ns |
| $NH_3$-N | 4.30 | 4.14 | 4.71 | 6.36 | 6.10 | 6.94 | 7.87 | 8.25 | 8.28 | 0.83 | * | ns | ns |
| DM Dig[c] | 733.3 | 660.0 | 586.6 | 720.0 | 640.0 | 646.6 | 760.0 | 600.0 | 626.6 | 2.13 | ns | * | * |

a. Urea (U) and molasses (M) levels as % of canola forage DM.
b. All data quoted as g/Kg DM except pH and ammonia (mg/ dl rumen fluid)
c. DM digestibility as g/Kg of canola silages DM.
ns = the effect is not significant ( $p> 0.05$ ).
* = the effects is significant ($p< 0.05$).

**Discussion**. It seems that the ammonia released as a result of bacterial hydrolysis of urea increased CP and $NH_3$-N in silages treated with urea. On the other hand, addition of molasses decreased NDF, ADF and DM digestibility and increased DM contents of the canola silages which could be related to the high DM content of the molasses used. The results of this study demonstrate that supplementing canola forage with urea can decrease silage quality by increasing silage pH and its N-NH3 concentration. So the application of urea in canola forage chemical treating is not suggested. Application of molasses for canola forage chemical treating can increase canola silage quality by decreasing silage NDF and ADF content. But application percentage of molasses is important because its high levels can decrease canola silage DM digestibility.

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# Effects of a bacterial inoculant on chemical composition and fermentation parameters of corn silage ensiled in a laboratory silo

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**Introduction** The use of lactic acid bacteria (LAB) as microbial inoculants in order to increase bacterial fermentation and decrease the pH of silage which in turn inhibit the growth of undesirable microbes and provide stable ensiling conditions has been well documented (McDonald, 1991). Although corn silage is used as major forage for dairy cattle at most commercial farms in Iran no information is available regarding the suitability of LAB for their use as inoculants in preparing corn silage. The objective of this study was therefore to determine the effects of the addition of a bacterial inoculant on chemical composition and fermentation characteristics of corn forage ensiled in 2 kg laboratory silos.

**Materials and methods** Corn (Zea mays L.) was planted on June 2005 and was harvested at dry matter (DM) of about 21% for ensiling in 2 kg plastic jars with or without a bacterial inoculant (control). The inoculant (LALSIL MSO1, LALLEMAND, France) contained $10^9$-$10^{10}$ CFU/g of each of *Lactibasillus plantarium* (MA18/5U) and *Propionibacterium acidipropionici* (MA26/4U) strains. The inoculant (2.5 g/l) was added to chopped corn forage (inoculated) which was then used to fill replicated jars containing lids that enabled excess gas release. The study followed a 2x6 factorial arrangement, in triplicate, to distribute each of the control and inoculated forages into 18 jars involving 6 ensiling times (1, 2, 3, 5, 15, 45 days or D) in triplicate. The air from each jar was removed by pressing each forage with a 5 kg metal bar before the jars were closed for storage in dark at about 20-25$^o$C. After relevant days post-ensiling, forage in each jar was sampled for determining DM, fermentation parameters (pH, buffering capacity, $NH_3$-N and VFA) and chemical compositions (CP, NDF, ADF and EE). The data were statistically compared by using GLM in Minitab for the effect of inoculation and days post-ensiling for significance (Sign) at $P < 0.05$.

**Table 1** Chemical composition (g/kg DM) and fermentation parameters of corn forage after 0 and 45 days of ensilage with or without bacterial inoculant

| | Control silage (C) | | Inoculated silage (I) | | Main effects SE (n=6) | Significance for main effects | |
|---|---|---|---|---|---|---|---|
| Parameters | Day 0 | Day 45 | Day 0 | Day 45 | | C v I | D0 v D45 |
| Dry matter (g/kg) | 209 | 184 | 210 | 194 | 0.18 | NS | *** |
| CP | 51.6 | 52.9 | 51.4 | 53.1 | 1.52 | NS | NS |
| NDF | 523 | 561 | 527 | 547 | 10.1 | NS | NS |
| ADF | 317 | 395 | 332 | 367 | 12.9 | NS | * |
| Ether extract (EE) | 12.3 | 11.5 | 10.9 | 8.9 | 0.81 | NS | NS |
| pH | 6.68 | 3.73 | 6.71 | 3.67 | 0.018 | NS | *** |
| Acetic acid (g/kg DM) | 5.6 | 59.3 | 5.1 | 60.9 | 0.70 | NS | *** |
| Buffering capacity | 12.8 | 66.9 | 13.5 | 69.8 | 1.2 | NS | *** |
| $NH_3$-N (g/kg total N) | 0.0 | 20.2 | 0.0 | 14.2 | 0.79 | * | *** |

NDF, neutral detergent fibre; ADF, acid detergent fibre; CP, crude protein; SE, standard error of mean;
*= P<0.05 &*** =P<0.001; NS= non significant

**Results** Due to the weather condition, the DM content of corn forage was very low at harvesting time. Table 1 shows that DM and pH reduced whereas acetic acid, buffering capacity and $NH_3$-N increased significantly ($P<0.001$) with ensiling (D0 v D45) of corn forage. NDF and ADF were also tended to increase with ensiling. The main effects of inoculation (C v I) were not significant for chemical composition and fermentation characteristics (except $NH_3$-N) of the forage. However, the inoculated silage showed better fermentation characteristics (lower pH and higher acetic acid and buffering capacity) than those of the control at D45 and all other sampling times The amount of propionic, butyric and isobutyric acids were undetectable in all samples. The inoculated forage showed significantly lower $NH_3$-N at all times including D45 ($P<0.05$) than the control which perhaps was an indication of reduced proteolysis due to better preservation of inoculated forage.

**Conclusions** Both the chemical composition and the fermentation data suggest that the microbial inoculant has the potential to provide a better condition for forage preservation during ensilage. However further studies are continued to explore the possibility of enhancing the efficacy of such inoculants to produce better quality high DM corn silage compared with the unusually low DM forage of this study.

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# Effect of acid hydrolysis steam pressure treatment on physico-chemical properties and bio-utilization of sugarcane bagasse

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**Introduction** fibrous materials such as sugarcane bagasse contain more than 600 g/kg of its dry matter in the form of cellulose and hemicellulose but its degradability is very poor. One of the main reasons for this depression in degradability is the presence of lignin which protects carbohydrates from being attacked by rumen microbes. The main effect of steam treatment is to increase feed intake, overall digestibility and gain which is achieved because of the extensive destruction of cell wall and hemicellulose hydrolysis which increases soluble sugars and makes cell wall more degradable (Karkoodi et al, 2006). The aim of this study was to assess the effect of acid hydrolysis steam pressure treatment on physico-chemical and bio-utilization of sugarcane bagasse.

**Material and methods** 1% Sulphuric acid solution was added to ground sugarcane bagasse to obtain samples of approximately 500 $gkg^{-1}$ DM content. Samples were put in stainless steel baskets and placed in reaction chambers. Steam treatment of samples carried out by direct injection of steam into the chamber and the samples were kept under a specific pressure and a period of time (reaction time) followed by its depressurization. A completely randomized design with a 3×3 factorial arrangement with three replicates was used to assess the effect of pressure (P) and reaction time (T) on hemicellulose (Hem) (Ternud et al, 1989) and water soluble sugar (WSS) (Dubois et al, 1956) content. Also, samples were incubated by 128 IU Cellulase Onozuka (EC: 3.2.1.4) for 48h and then reducing sugar (R.Sug) content was determined (Nelson, 1944) and Accessible pore volumes (APV) for 8, 12, 51, 110, 270 and 550 $A^o$ probe molecules (Stone and Scallan, 1968) were measured according to unabsorbed sugar concentration of samples. Data from 3 pressures including 14, 17 and 20 atm and 3 reaction times including 120, 180 and 240 seconds were analyzed then means were tested according to duncan's least range test while all means were compared with untreated bagasse (control ) by Dunnett's test.

**Results** Results are presented in Table1 showed that steam-pressure treatment significantly decreased Hem content and increased R.Sug concentration after enzymic hydrolysis and also, all values for APV improved in comparison with untreated sugarcane bagasse ($P<0.01$). Increasing pressure resulted in considerable improvement of all parameters except for WSS ($P<0.01$). Similar results were obtained by increasing the reaction time ($P<0.01$) except for WSS, R.Sug, $APV_{12}$ and $APV_{270}$.

**Table 1** Hem and WSS content, R.Sug and APV of steam treated samples.

| $P^1_{(atm)}$ | $T_{(second)}$ | Hem | WSS | R.Sug | $APV_8$ | $APV_{12}$ | $APV_{51}$ | $APV_{110}$ | $APV_{270}$ |
|---|---|---|---|---|---|---|---|---|---|
| control | | 239.45 | 150.90 | 64.83 | 0.70 | 0.32 | 0.23 | 0.15 | 0.07 |
| | 120 | $180.50^{a2}$ | $217.17^{a}$ | $86.58^{d}$ | $0.97^{f}$ | $0.80^{d}$ | $0.59^{e}$ | $0.34^{e}$ | $0.16^{e}$ |
| 14 | 180 | $166.53^{a}$ | $241.43^{a}$ | $94.86^{d}$ | $1.20^{e}$ | $0.87^{d}$ | $0.64^{e}$ | $0.43^{d}$ | $0.19^{de}$ |
| | 240 | $136.19^{b}$ | $266.94^{a}$ | $100.63^{bcd}$ | $1.22^{e}$ | $0.91^{cd}$ | $0.66^{de}$ | $0.47^{d}$ | $0.21^{cde}$ |
| | 120 | $135.78^{b}$ | $236.44^{a}$ | $101.11^{cd}$ | $1.21^{e}$ | $0.94^{c}$ | $0.78^{d}$ | $0.53^{d}$ | $0.22^{cde}$ |
| 17 | 180 | $106.63^{c}$ | $278.95^{a}$ | $104.46^{c}$ | $1.40^{d}$ | $1.14^{bc}$ | $0.82^{cd}$ | $0.59^{cd}$ | $0.24^{cd}$ |
| | 240 | $90.10^{d}$ | $311.60^{a}$ | $117.52^{ab}$ | $1.49^{c}$ | $1.64^{b}$ | $0.87^{c}$ | $0.61^{c}$ | $0.25^{bcd}$ |
| | 120 | $114.58^{c}$ | $274.20^{a}$ | $109.88^{abc}$ | $1.60^{bc}$ | $1.32^{ab}$ | $0.98^{bc}$ | $0.74^{b}$ | $0.29^{abc}$ |
| 20 | 180 | $91.60^{d}$ | $319.07^{a}$ | $123.53^{a}$ | $1.64^{b}$ | $1.35^{a}$ | $1.06^{b}$ | $0.79^{ab}$ | $0.31^{ab}$ |
| | 240 | $60.10^{e}$ | $285.91^{a}$ | $113.60^{ab}$ | $1.80^{a}$ | $1.47^{a}$ | $1.24^{a}$ | $0.91^{a}$ | $0.33^{a}$ |
| s.e.m | | 0.423 | 3.275 | 0.448 | 0.038 | 0.081 | 0.056 | 0.051 | 0.025 |

[1] P: pressure, T: Reaction Time, Hem: Hemicellulose ($gkg^{-1}$ DM), WSS: Water Soluble Sugar ($gkg^{-1}$ DM), R.Sug: Reducing Sugar ($gkg^{-1}$ DM), $APV_8$, $APV_{12}$, $APV_{51}$, $APV_{110}$ and $APV_{270}$ accessible pore volumes for 8, 12, 51, 110 and 270 $A^o$ probe mollecules ($mlg^{-1}$).[2] Within columns, means with different superscripts differ ($P<0.05$).

**Conclusions** Acid hydrolysis of sugarcane bagasse significantly decreased Hem and increased WSS content and its bio-utilization by enzymes because of the more cell wall destruction, but because the acid catalyst assisted cell wall hydrolysis, steam treatment could have better bio-utilization even in shorter reaction times (180s vs. 240s).

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# Chemical composition and *in vitro* digestibility of nine plant species from Semnan rangeland for camel in Iran

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**Introduction** In Iran, Javan (2001) has reported the digestibility some arid rangelands plants by bovine rumen liquor. The determination of *in vivo* digestibility of wheat straw implies that camel apparently digested poor quality roughages more than cattle and sheep (Cianci *et al.,* 2004). Therefore, It is required to measure the *in vitro* digestibility of herbages by camel rumen liquor. In province of yazd, nutritive value of 11 different plant species for camel were determined (Towhidi, 2007). The objectives of the current study were to determine 1) the chemical composition, gross energy of the most consuming plant species from rangeland of Semnan province including *Seidlitzia rosmarinu,, Tamarix tetragyna, Tamarix strica, Halostachys spp,, Saudea fruticosa., Alhagi camelorum, Haloxylon ammondendron., Salsola arbescola, Hammada salicornica* and, 2) *in vitro* digestibility of the plants by camel rumen liquor

**Material and methods** The plants samples were collected in autumn 2002 based on stratified random sampling from the rangelands of Semnan province in Iran . 30 samples from the browsing parts prepared, pooled and dried at room temperature and milled. Chemical composition of samples including dry matter (DM), crude fibre (CF), Neutral detergent fibre (NDF), acid detergent fibre (ADF), ether extract (EE), total ash (TA), macro elements (Ca, P, Mg, K), and gross energy (GE) were analyzed by standard methods (Abdel-Gawad *et al.,* 1998)) in Animal Science Research Institute, Karaj. To determine the digestibility of the plants, four mature male camels were fitted with fistula in the rumen under anaesthesia. The *in vitro* digestibility was measured in duplicate by camel rumen liquor and pepsin in Tilley and Terry method (1963). Percentage of dry matter digestibility (%DMD), organic matter digestibility (%OMD), and organic matter digestibility in dry matter (% DOMD) were calculated.

**Results** The results of determination of chemical composition, gross energy and digestibility are presented in table 1. The highest CP (10.7%), and the lowest NDF (38.6%) and ADF (24.6%) were related to *Alhagi camelorum* and *Haloxylon ammondendron* respectively. The lowest CP (5.5%), and the highest NDF (69.4%) and ADF 46.2%) were related to *Hammada salicornica* and *Halostachys spp* respectively. The highest and the lowest %DOMD were related to *Haloxylon ammondendron* (70.18) and *Saudea fruticosa* (18.53), respectively.

**Table 1** Chemical composition and *in vitro* digestibility of nine plant species

| Scientific name of plants | DM% | CP% | NDF% | ADF% | TA% | EE% | GE( Mj/Kg) | Ca% | P% | Mg% | K% | Na% | %DOMD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Seidlitzia rosmarinus* | 93.6 | 7.9 | 60.5 | 38.3 | 24.5 | 0.5 | 14.465 | 1.38 | 0.15 | 18.7 | 0.40 | 1.20 | 29.81 |
| *Tamarix tetragyna* | 94.7 | 6.8 | 51.2 | 36.2 | 9.6 | 1.7 | 16.525 | 1.30 | 0.18 | 20.0 | 0.40 | 1.1 | 28.09 |
| *Tamarix strica* | 95.4 | 8.0 | 56.8 | 39.0 | 8.0 | 1.7 | 17.139 | 1.20 | 0.13 | 21.5 | 0.39 | 1.19 | 35.01 |
| *Halostachys spp.* | 95.5 | 7.7 | 69.4 | 46.2 | 9.1 | 1.5 | 16.385 | 1.02 | 0.20 | 16.0 | 0.24 | 0.99 | 21.61 |
| *Saudea fruticosa* | 93.9 | 7.9 | 67.4 | 40.0 | 9.9 | 1.0 | 17.555 | 1.20 | 0.19 | 35.0 | 0.35 | 1.03 | 18.53 |
| *Alhagi camelorum* | 95.1 | 10.7 | 47.2 | 35.8 | 11.2 | 0.3 | 16.558 | 0.93 | 0.17 | 60.0 | 0.25 | 1.35 | 32.15 |
| *Haloxylon ammondendron* | 94.3 | 8.6 | 38.6 | 24.6 | 28.7 | 1.4 | 12.896 | 0.93 | 0.07 | 77.5 | 0.19 | 1.38 | 46.91 |
| *Salsola arbescola* | 94.5 | 6.8 | 48.4 | 30.8 | 22.0 | 1.4 | 14.206 | 1.25 | 0.06 | 132.0 | 0.23 | 1.04 | 42.88 |
| *Hammada salicornica* | 95.3 | 5.5 | 61.6 | 45.0 | 19.1 | 1.4 | 16.150 | 1.05 | 0.02 | 81.0 | 0.26 | 0.77 | 20.76 |

**Conclusions** Our data are the first report about the *in vitro* digestibility of the some herbage in province of Semnan which obtained by camel rumen liquor. There were no consistent patterns between the chemical composition with the digestibility of the plants. For further study, it is suggested to investigate the relationship between the other contents of plants, for example lignin with the digestibility.

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# The concentration of oxygen and its depletion in bovine red clover-boluses

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**Introduction** The enzyme polyphenol oxidase (PPO) may be responsible for increases in both dietary-nitrogen utilisation and the concentration of polyunsaturated fatty acids in ruminant products when animals eat red clover (Lee *et al.* 2004). The enzymatic reaction requires the presence of oxygen and therefore is assumed to be inhibited in the anaerobic rumen. However, no information exists as to the concentration of oxygen in red clover-boluses and whether this could sustain the aerobic enzyme's activity in the reticulo-rumen. This study investigated the concentration of oxygen in red clover-boluses measured *in vitro* or *in vivo* in the rumen of two rumen-fistulated cows.

**Materials and methods** The rumen of a Holstein × Friesian dairy cow fitted with a rumen cannula was emptied. The cow was then offered freshly cut red clover and the freshly ingested bolus caught at the oesophageal orifice in the reticulo-rumen. This was immediately transferred to an air-tight jar containing de-oxygenated water maintained at 39°C. A dissolved oxygen probe (Orion Research Inc, MA, USA, calibrated according to the manufacturer's instructions) was inserted into the bolus and the oxygen concentration measured at 30 second intervals over a time course of ten minutes. This procedure was then repeated twice more (n=3) and the data analysed using a repeated measurement ANOVA (Genstat 8.1, Lawes, Agricultural Trust, 2005). A second set of red clover boluses (n=3) were then incubated in strained rumen fluid and the oxygen concentration measured as before. *In vivo* measurements were then taken using two rumen fistulated dairy cows. The oxygen probe was positioned inside the reticulo-rumen by the oesophageal orifice and a reading taken as the initial oxygen concentration. The animals were then allowed to eat for one mouthful before the red clover was removed. The time was recorded between swallowing and observing the oxygen peak as the bolus entered the rumen. When a stable oxygen reading was observed this was recorded as the initial value and the animal offered the red clover once again for one more mouthful. This was repeated several times (cow 1, n=6; cow 2, n=4).

**Results** Fig. 1. shows the oxygen depletion from the red clover boluses incubated in de-oxygenated water, which reached completion after 5 minutes. When the red clover bolus was immersed in strained rumen fluid the oxygen disappeared within 2 minutes (Fig. 2). In the *in vivo* study there were no significant differences between cows, the initial concentration of oxygen in the reticulo-rumen was negligible. The entrance of a bolus into the rumen caused a peak in oxygen concentration of 0.28 (±0.199) mg $O_2$/l, 5.9 (±0.28) seconds after swallowing, returning to the initial oxygen concentration within 6.4 (±1.94) seconds.

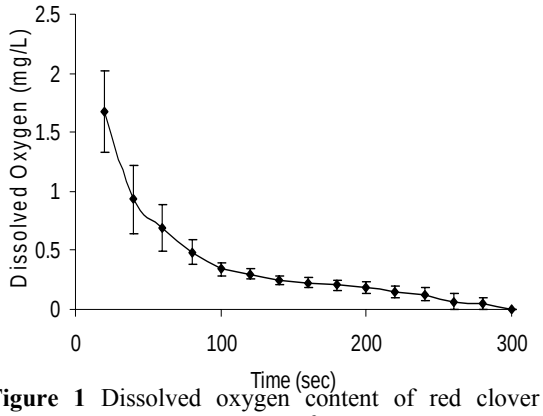


**Figure 1** Dissolved oxygen content of red clover boluses incubated in water at 39°C n=3 (± SEM)

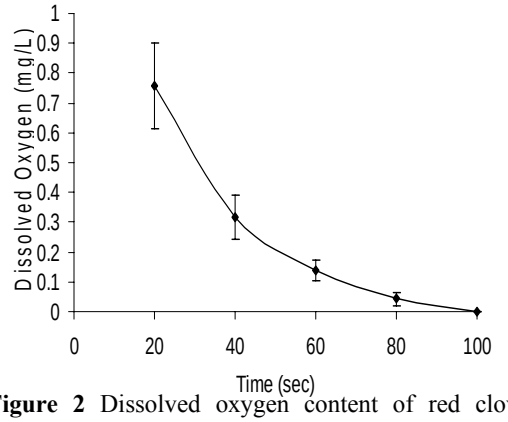


**Figure 2** Dissolved oxygen content of red clover boluses incubated in rumen liquor at 39°C n=3 (± SEM)

**Conclusions** The results from the current study agree with the finding of Lee et al. (2006) with grass boluses, although the red clover resulted in a faster depletion of oxygen than the grass boluses and a lower concentration of oxygen *in vivo*. Although the bolus alone resulted in oxygen depletion, it appears to be largely due to the oxygen scavenging nature of the microbes in the rumen fluid as oxygen disappeared from the boluses immersed in rumen fluid within 2 min but some oxygen was still present up to 5 min when immersed in de-oxygenated water. Given these results it is apparent that any occurrence of PPO activity during grazing of red clover would be largely confined to the period of mastication and that the amount of oxygen brought in from the boluses would be rapidly scavenged.

**Acknowledgement** Department for Environment Food and Rural Affairs for funding the research.

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# Gestation length in first calving Aberdeen Angus x Limousin and Limousin x Aberdeen Angus heifers mated using a synchronised artificial insemination programme

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**Introduction** Recent changes to beef production subsidy schemes in the UK has focussed attention on the fertility and reproductive output of suckler beef systems in an attempt to ensure future financial sustainability. Adoption of modern cattle breeding technologies may be more common in the future than they have been in the past as a result of this renewed focus on commercial profitability rather than subsidy reliance. However, relatively little information is available on the biological parameters encountered when modern breeding approaches are employed within commercial crossbred suckler beef management systems in the UK. Previous work (Penny, 2005) has detailed the development of either a triple or double synchronised artificial insemination (AI) programme suitable for use with both multiparous suckler cows and first calving heifers managed as a commercial spring calving suckler herd. The objective of the current report is to detail the effects of breed type and calf sex on average gestation length (GL) in first calving crossbred suckler replacement heifers when mated using a synchronised AI programme.

**Materials and methods** Data on GL was gathered from heifers that calved for the first time over four spring calving seasons (2003, 2004, 2005 and 2006) from a two-way rotational crossbred suckler cow herd maintained at the Scottish Agricultural College (SAC) and managed under commercial conditions. Heifer breed types were either Aberdeen Angus cross Limousin (AAx) or Limousin cross Aberdeen Angus (LIMx) according to the recipricol nature of the two-way cross breeding programme within the herd. All first calved heifers across all four years were managed in the same way and had been mated according to a synchronised AI programme as described by Penny, (2005). Each heifer was given two opportunities to conceive using the synchronised AI protocol and were due to calve during March – May of each year. All AAx heifers were always sired using a pure LIM sire whilst all LIMx heifers were always sired using a pure AA sire to maintain the two-way rotational crossbred suckler cow herd genotype. No heifer was every allowed access to mating by natural service so GL could be determined accurately for each animal. GL was determined as the days elapsed from the last recorded synchronised AI mating to the actual date of calving the following spring. A total of 48 and 43 data sets where GL was determined were available for the AAx and LIMx heifer genotypes respectively. GL data was statistically analysed using the residual maximum likelihood procedure (REML) in Genstat 5 according to a 2 x 2 x 4 (heifer breed x calf sex x year) randomised continous block design.

**Results** Observed GL ranged from 274 – 297 days and from 269 – 292 days in AAx and LIMx heifers respectively. No significant effect of year was seen in the statistical analysis so only GL data for the heifer genotype by calf sex interaction along with the respective average values are shown in Table 1. Neither calf sex nor heifer genotype were significant factors influencing GL in their own right. However, within this dataset male calves sired by pure LIM sires from AAxLIM heifers had a significantly longer GL (287 days) compared with female calves sired by AA sires from LIMxAA heifers (283 days).

**Table 1** Gestation length (days) in AAxLIM and LIMxAA heifers according to sex of calf.

| Heifer breed | AAxLIM | LIMxAA | | |
|---|---|---|---|---|
| Sire breed | LIM | AA | s.e.d | Significance |
| Calf sex | | | | |
| MALE | 287[a] | 285[ab] | | |
| FEMALE | 286[ab] | 283[b] | 1.7 | * |
| Breed type average | 286 | 284 | 1.2 | |
| | MALE | FEMALE | | |
| Calf sex average | 286 | 284 | 1.2 | |

Values not sharing common superscripts differ significantly ($P<0.05$).

**Conclusions** Data on GL in commercial crossbred suckler animals is difficult to obtain since most suckler cows and heifers are mated using natural service and actual conception dates are not known. However, the use of a synchronised AI programme rather than natural service in the SAC resource herd reported here, has allowed both the ranges and average GL in typical crossbred suckler heifer replacements sired with either pure AA or pure LIM sires to be made available to the wider UK beef industry.

**Acknowledgements** SAC receives financial support from SEERAD.

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# Kale-based out-wintering systems for weaned Aberdeen Angus cross and Limousin cross steers during a winter store period

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**Introduction** Since de-coupling of CAP subsidy schemes from actual animal production, lowering the costs of finishing cattle production systems is one of the key challenges facing the beef industry. One of the largest costs associated with finished cattle production from spring-born suckled calves is the costs of over-wintering the weaned steer calf once the suckling phase is over. The capital tied up in buildings is a major component of these costs in countries such as the UK where the relatively harsh winter environments have traditionally led to many weaned suckled calves being housed and fed conserved forages over the winter months to ensure adequate performance. Previous work (Hyslop *et al*, 2006) has shown that under appropriate field conditions, non-lactating, spring calving suckler cows can be out-wintered on both grazed kale or turnip based-systems with an acceptable degree of animal performance. The objective of the current study was to determine liveweight (LW) changes in weaned spring-born suckled calf steers when managed outdoors on kale-based grazing systems during a short winter feeding period and to assess the dirtiness of steers hides at the end of the kale grazing period.

**Materials and methods** After they had been weaned in early November 2005, a total of 45 spring-born suckled calf steers were allocated to one of 3 winter feeding groups on the basis of steer breed type and LW. Steer breed types used were either Aberdeen Angus cross (AAx) or Limousin cross (LIMx) where 6 AAx and 9 LIMx steers were allocated to each of the 3 feeing groups. The 3 winter feeding groups were as follows:- kale grazing + straw, (KAL-STR), kale grazing + silage, (KAL-SIL) and a housed control group where indoor diets were based on *ad libitum* whole crop cereal silage and 2 kg/h/day of a cereal based concentrate (HOUSED). Both the KAL-STR and KAL-SIL groups were outwintered and given access to individual plots of a standing kale crop (var: *Maris Kestrel*) using a strip grazing system behind an electric fence which was moved daily. The KALE-STR groups also had *ad libitum* access to bales of spring barley straw whilst the KAL-SIL group had *ad libitum* access to bales of grass silage, also both behind the electric fence. Each of the out-wintered steer groups also had access to their own grass run-back area and free access to a mineral supplement. Steers in the HOUSED group were kept indoors in a straw bedded yard and fed the whole crop cereal based diet on a daily basis. Individual steer LW measurements were determined at the start (22/11/05) and end (17/01/06) of the short winter feeding period respectively whilst hide dirtiness score (DS) was determined on a 1-5 scale according to the method described by the Meat Hygiene Service (1997) where DS1 = dry & clean; DS2 = dry with light faecal/dirt contamination; DS3 = dry/damp with significant faecal/dirt contamination; DS4 = dry/damp with heavy faecal/dirt contamination and DS5 = wet with heavy faecal/dirt contamination . LW changes were determined by difference and all data were statistically analysed using the residual maximum likelihood procedure (REML) in Genstat 5 according to a 3 x 2 randomised continous block design with 3 treatment groups (KAL-STR, KAL-SIL and HOUSED) and 2 steer breed types (AAx and LIMx).

**Results** No significant differences between steer breed types were found so only feeding group averages are given for steer LW, daily liveweight gain (DLWG) and dirtiness score values in Table 1. No significant differences were seen in start or end LW figures but DLWG was lower ($P<0.05$) and dirtiness score was higher ($P<0.001$) for the kale based out-wintering systems compared to the housed control group.

**Table 1** LW (kg), DLWG (kg/d) and dirtiness scores (1-5 scale) in out-wintered spring-born suckled steers.

| | KAL-STR | KAL-SIL | HOUSED | s.e.d. | Sig. |
|---|---|---|---|---|---|
| Start LW (kg) | 336 | 338 | 334 | 7.5 | |
| End LW (kg) | 357 | 363 | 367 | 7.5 | |
| DLWG (kg/d) | 0.37[a] | 0.44[a] | 0.60[b] | 0.058 | * |
| Dirtiness score (1-5 scale) | 2.42 [a] | 2.50 [a] | 1.44 [b] | 0.215 | *** |

**Conclusions** Under appropriate field conditions, weaned, spring-born suckled calf steers can be out-wintered on both KAL-STR and KAL-SIL systems with an acceptable degree of LW gain during a short winter store feeding period. Observations on steer hide dirtiness score indicate that more work may be needed to investigate the dirtiness of steers kept outdoors on kale based grazing systems, especially if they are to be slaughtered from this system. Further detailed studies are required to establish steer performance on kale based out-wintering systems over much longer winter feeding periods.

**Acknowledgements** QMS provided funding for this work. SAC receives financial support from SEERAD.

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# Effect of encapsulated conjugated linoleic acid isomers on carcass composition of beef steers

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**Introduction** Dietary supplements of a mixture of CLA isomers have been shown to reduce body fat accretion in mice and pigs with the trans-10, cis-12 isomer probably being responsible for the anti-lipogenic effects. The efficacy of calcium salts of a mixture of CLA methyl esters containing trans-10, cis-12, on milk fat synthesis, is relatively low (Bernal-Santos *et al*., 2003) and reported lack of effects on growth may be due to low efficacy and the short duration of CLA supplementation, during the last 32 or 60 days before slaughter, and the relatively low level of trans-10, cis-12 CLA fed. Shingfield *et al* (2004) demonstrated the potential of rumen protected supplements of a mixture of CLA isomers to reduce milk fat content and improve the energy status of dairy cows during early lactation. The current experiment used a lipid-encapsulated supplement containing equal amounts of cis-9, trans-11 and trans-10, cis-12 CLA methyl esters to study the effects on the performance and carcass characteristics of Limousin steers during a 100d finishing period.

**Materials and methods** Forty Limousin steers, at the University of Reading beef unit, had *ad libitum* access to a basal diet (forage (equal parts grass and maize silage):concentrate ratio 60:40 on a dry matter basis). On reaching a live weight of 425 kg, each steer was allocated at random to one of 4 experimental treatments in a 2 (grass or maize silage, forage:concentrate ratio 60:40 on a DM basis) x 2 (control or CLA lipid supplement) factorial design . The lipid-encapsulated CLA supplement was prepared by binding methyl esters of CLA (Luta-CLA 60 supplied by BASF, Germany) to a silica matrix and coating this complex with hydrogenated soybean oil by Balchem Encapsulates (New Hampton, New York). It was expected that the intake of trans-10, cis-12 CLA would be circa 75 g, significantly higher than in earlier studies. Major fatty acids in the CLA mix (g/100 g total fatty acids) were: C16:0, 5.26; C18:0, 4.12; *cis*-9 C18:1, 25.66; C18:2*n*-6, 1.99; *cis*-9, *trans*-11 CLA, 29.32; *trans*-10, *cis*-12 CLA, 29.09.
After 100d on diet steers were slaughtered at the University of Bristol. At 48h post-mortem, carcass loin muscle and fat widths and depths were measured, a sample joint from the forelimb was taken for dissection into constituent tissues and a sample of m. *longissimus thoracis* was removed for fatty acid analysis.

**Results** Feeding CLA increased levels of trans-10, cis-12 CLA 10 to 16-fold in muscle (Table 1) and 11 to 12-fold in adipose tissue. Concentrations of cis-9, trans-11 CLA were increased up to 2-fold in muscle and 1.5-fold in adipose tissue. Although there were numerical reductions in fat content caused by CLA treatment, only the effect on kidney fat was statistically significant.

**Table 1** Carcass characteristics and muscle CLA content of steers fed a grass or maize silage diet with or without CLA supplementation

| | diet fed | | | | | |
|---|---|---|---|---|---|---|
| attribute | Grass | Maize | Grass+CLA | Maize+CLA | sed | sig |
| Cold side wt (kg) | 143 | 149 | 143 | 146 | 3.3 | ns |
| Kidney&Cod fat(kg) | 13.9[a] | 13.1[a] | 11.3[b] | 10.4[b] | 1.26 | 0.03 |
| loin muscle at 10th rib | | | | | | |
| length a (mm) | 137 | 141 | 140 | 142 | 3.3 | ns |
| width b (mm) | 58 | 62 | 59 | 60 | 2.5 | ns |
| fat depth (mm) | 11.4 | 10.7 | 9.9 | 8.0 | 2.6 | ns |
| loin muscle area ($mm^2$) | 726 | 784 | 729 | 751 | 29.1 | ns |
| ***forelimb dissection*** | | | | | | |
| total muscle (kg) | 12.1 | 12.5 | 12.1 | 12.8 | 0.32 | ns |
| % muscle | 62.5 | 62.6 | 63.2 | 62.8 | 0.47 | ns |
| total fat (kg) | 2.5 | 2.70 | 2.4 | 2.6 | 0.12 | ns |
| % fat | 13.1 | 13.7 | 12.8 | 12.8 | 0.58 | ns |
| total bone (kg) | 4.7 | 4.7 | 4.6 | 4.9 | 0.12 | ns |
| % bone | 24.4 | 23.7 | 23.8 | 24.1 | 0.48 | ns |
| mg/100g muscle | | | | | | |
| cis-9, trans-11 CLA | 9.7 | 11.7 | 19.3 | 15.2 | 2.06 | *** |
| trans-10, cis-12 CLA | 0.42 | 0.58 | 6.97 | 5.75 | 0.66 | *** |

**Conclusions** Feeding large amounts of CLA isomers to beef cattle increased their deposition in muscle substantially but body fat reduction was so small as to make this an unsuitable technology for finishing cattle.

**Acknowledgements.** This work was supported by the Department for Environment Food and Rural Affairs.

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# Venous Blood Gas in Holstein steers fed diets with different concentrate to lucerne hay ratios

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**Introduction** An arterial or venous blood gas is a clinical tool for determining pulmonary and metabolic status. Arterial Blood Gas (ABG) or Venous Blood Gas (VBG) methods provide a direct measurement of partial blood pressures of carbon dioxide ($PaCO_2$) and oxygen ($PaO_2$), hydrogen ion activity (pH), total hemoglobin (Hbtotal), oxyhemoglobin saturation ($HbO_2$) and bicarbonate ion concentration ($HCO_3^-$). Most blood tests are done on a sample of blood taken from a vein due to: 1-Collecting blood from an artery is more painful than collecting it from a vein because the arteries are deeper and have more nerves, 2- Artery may be inaccessible due to periarterial tissues (overlying muscle, connective tissue, or fat). In ruminants, feeding diets high in grain and other highly fermentable carbohydrates increases the risk of ruminal and blood acidosis. Although ruminal pH varies considerably within a day, cows possess a highly developed system to maintain ruminal pH within a physiological range. However, if the acid production from fermentation is more than the system can buffer, ruminal pH compensation fails and it may drop drastically (Marie Krause & Oetzel, 2005). The importance of arterial or venous blood gas measurements in the diagnosis of ruminal acidosis is prevented some health problems such as ruminal parakeratosis, erosion and ulceration of the ruminal epithelium (Garry, 2002), laminitis, sole abscesses and sole ulcer (Nocek, 1997). The objective of the present experiment was to investigate the effect of diets providing different concentrate: lucerne hay ratios on venous blood gas in Holstein steers.

**Materials and methods** Four Holstein steers (300 ± 15 kg, body weight) were adapted to experimental diets for one week. Steers fed 7 kg of DM of diets differing in concentrate (155 g CP $kg^{-1}$ of DM; 30% maize, 34% barley, 8% soybean meal, 5% sugar beet pulp, 10% wheat bran, 12% cottonseed meal, 0.3% $CaCo_3$, 0.5% mineral and vitamin premix, 0.2% salt ) to lucerne hay (155 g CP $kg^{-1}$ of DM ) ratios as 60:40, 70:30, 80:20, and 90:10 in a 4×4 Latin square design (28 days of each period). Steers were housed in individual pens, and fed the experimental diets as total mixed ration twice daily at 0800 and 2000 h. Animal had access to drinking water at the all time. At day 25 of each period of the experiment, blood samples were taken from Jugular vein after 4 h morning feeding. Samples were analyzed for VBG by Automatic blood gas system (AVL 995, Switzerland). Data were analyzed using the GLM procedure of SAS (y = Mean + Treatment + Animal + Period + residual) and the means compared by the Duncan test ($P < 0.05$).

**Results** Venous blood gas values are shown in the Table 1. Results indicated that the blood pH and VBG values were not significantly influenced by the diets. However, blood pH decreased from 7.41 (T1) to 7.37 (T4). When level of concentrate was increased.

**Table 1** Venous blood gases in Holstein steers fed diets differing in concentrate: lucerne hay ratios

| Item | Concentrate: lucerne hay ratio[1] | | | | Treatment effect | |
|---|---|---|---|---|---|---|
| | 60:40 | 70:30 | 80:20 | 90:10 | SEM[2] | *P* |
| Blood pH | 7.41 | 7.39 | 7.40 | 7.37 | 0.02 | 0.24 |
| $Paco_2$ (mmHg) | 53.80 | 61.58 | 56.55 | 57.13 | 3.94 | 0.6 |
| $Pao_2$ (mmHg) | 41.73 | 40.13 | 41.35 | 43.15 | 2.87 | 0.9 |
| $Hco_3^-$ (mEq/lit) | 32.38 | 34.80 | 33.58 | 31.85 | 1.49 | 0.54 |
| $O_2$ saturation (%) | 71.20 | 65.58 | 71.33 | 68.35 | 4.66 | 0.79 |

1: Values were reported as the mean of four sampling periods.
2: When the difference between means is greater than two times the SEM, it is considered as significant ($P < 0.05$).
3: SEM= Standard Error of Mean

**Conclusions** The results of the present study demonstrated that the increasing of concentrate in the diets of Holstein steers did not significantly affect blood pH. Results of the present study indicated that blood $HCO_3^-$ (mEq/lit) and $PaCO_2$ (mmHg) did not significantly changed when steers fed high concentrate diets. Therefore, it was concluded that the increasing of concentrate from 60 to 90 percent could not cause a mixed metabolic acidosis in steers.

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## Comparison of effects of different fat sources and their blend on broiler performance

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**Introduction** Arian broiler is one of the most important strains in Iran. There are many fat sources in poultry nutrition at Iran and other countries. These fat sources have different effects on broiler performance. Various fatty acids absorbed separately and differently in chicken lumen. Fats, especially linoleic acid have main effects on microbial flora at chicken (Pesti *et al*., 2002). Meanwhile fat sources have important share in costs of diet formulation. The aim of current experiment was to evaluate the effects of different fat sources and their blend on broiler performance.

**Materials and methods** One experiment with Arian male broilers was conducted using completely randomized design (CRD). The isocaloric (ME=12.12, 12.33 and 12.54 MJ/kg in starter, grower and finisher periods respectively) and isonitrogenous diets were fed ad libitum, but fat source of these diets were different. Fat sources in eight treatments were (1) 4% soybean fatty acid, (2) 4% poultry fat, (3) 4% bovine tallow, (4) 4% soybean fatty acid and tallow, (5) 4% soybean fatty acid and poultry fat, (6) 4% poultry fat and tallow, (7) 4% soybean fatty acid and poultry fat and tallow, and (8) control diet without additive fat. These diets applied in starter (7-21d), grower (21-42d) and finisher (42-49d). Eight experiment diets were fed in triplicate to the 1-week-old birds for a period of 6 weeks, after which body weight gain, feed intake, ME efficiency etc were recorded. ME for poultry fat calculated using Sibbald method (ME= 29.782 MJ/kg). At first week, it was used a basal diet without additive fat. Data were analyzed by two-way ANOVA and Duncan multiple range test using SAS.

**Results** From obtained analyzed results, it was showed that body weight gain significantly increased by adding fats to diets in compared to control diet ($P<0.05$). For consumed feed, diet containing poultry fat and tallow had significant different compared to control diet ($P<0.05$). Feed conversion ratio in diets containing soybean fatty acid, and soybean fatty acid and poultry fat blend, were better than blend of poultry fat and tallow, tallow as well as control groups significantly ($P<0.05$). The percentage of abdominal fat and liver showed no significant different between treatments ($P>0.05$). Poultry fat increased significantly pancreas percentage in comparing to soybean fatty acid ($P<0.05$). The blend of soybean fatty acid and poultry fatty acid had significantly less heart percentage comparing with the blend of soybean fatty acid and tallow ($P<0.05$). The blend of poultry fat and soybean fatty acid, the blend of soybean fatty acid and tallow, the blend of poultry fat and tallow and tallow showed significantly less gall bladder percentage than poultry fat ($P<0.05$). Furthermore in Figure 1, the consumed ME for per Kg weight gain in starter, grower, finisher and total periods are shown.

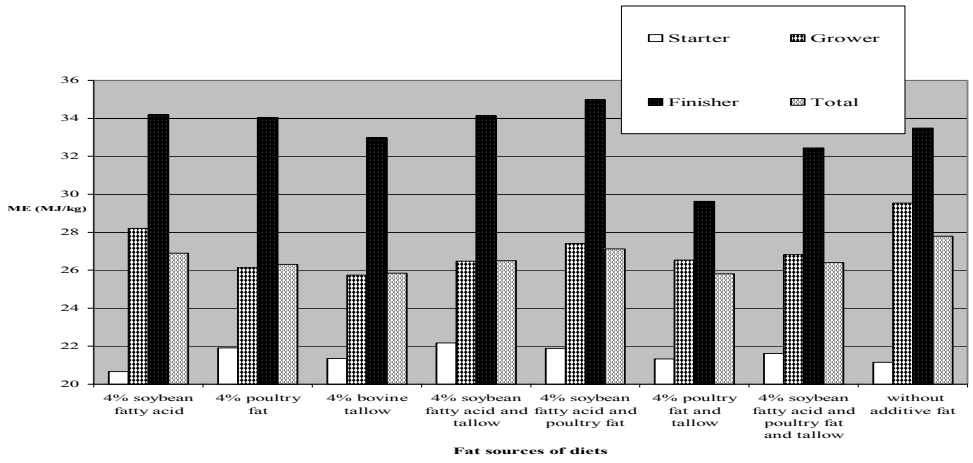


**Figure 1** Metabolizable Energy efficiency (MJ/kg gain) in different periods

**Conclusions** Obtained results showed that the blend of poultry fatty acid + soybean fatty acid, poultry fat and soybean fatty acid in broiler diets had suitable effect on broiler performance. Treatment containing higher ratio of unsaturated-saturated fatty acids had higher feed efficiency. Crespo and Estreve-Garcia (2002) obtained similar results. Meanwhile fat source contain soybean oil can improve digestibility and production index (Crespo and Estreve-Garcia, 2002).

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# Energy balances of beef tallow and soyabean oil in broiler chickens

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**Introduction** From previous reports indicate that broiler chickens fed diets enriched with polyunsaturated fatty acids have less abdominal fat or total body fat (Sanz et al., 1999) deposition than do broiler chickens fed diets containing saturated fatty acids. In general, body fat accumulation may be considered the net result of the balance among dietary absorbed fat, endogenous fat synthesis (lipogenesis) and fat catabolism via β-oxidation (lypolysis). Thus, if the amount of absorbed fat is the same, lower body fat deposition may be attributed to increased fat catabolism or diminished endogenous fatty acid synthesis or to both process. Differences in lipid oxidation rates seem to be the main mechanism involved in this effect. Supplementation of unsaturated fatty acids in diets can be enhanced fat catabolism and reduced fatty acid synthesis were reported to occur in rats fed polyunsaturated fatty acid rich in diets compared with rats fed diets enriched with saturated fatty acids (Crespo and Esteve-Garcia, 2002). Polyunsaturated fatty acids rich in diet effect on fat absorption were increased. In, contrast amount of abdominal fat deposition was decreased. It may be that the polyunsaturated fatty acid was higher rate fat oxidation or lower rate of fatty acid synthesis or both. The present study was undertaken to determine the effect of amount of beef tallow versus soybean oil absorbed is the same on energy intake, energy excretion, energy retention and energy expenditure of broiler chickens.

**Materials and methods** A total of 50 7-d-old male Arbor Acres broiler chicks were randomly distributed into 25 birds per treatment were kept in individual cages. Feed was provided restricted feeding in form of meal at 80% of feed intake. Birds were freely to clean water. The experimental diets were formulated to contained 18%CP. Fat in diets was calculated to digestibility is the same, which calculated by previous experiment information (supplemented between tallow 3.00%, BT and soybean oil 2.79%, SBO). Ingredients composition of both diets consisting tapioca starch 45.25%, soybean meal 41.05%, rice bran hull 4.00%, di-calcium phosphate 3.87%, lime stone 0.50%, DL-methionine 0.30%, L-lysine hydrochloride 0.25%, sodium chloride 0.51% and premixed 1.00%. Every day (7-28 days of age) were collected to excreta for analysis (apparent fat digestibility, fatty acids digestibility). Bomb calorimetry analysis for determined to the gross energy content in the diets, homogenised of whole carcasses and feces for calculated energy balance. Energy stored in the body was determined as total energy at the end of the 21 days feeding period minus the energy in the body at the beginning (=mean body weight x energy content) of the 21 days feeding period. Energy expenditure was calculated as the difference between the energy intake and energy stored and excreted in the excreta. (energy expenditure=energy intake – energy stored in the body – energy in excreta)

**Table 1** Influence of beef tallow and soybean oil on energy balance

| Item | Fat Types | | SEMs | P-Values |
|---|---|---|---|---|
| | BT | SBO | | |
| Energy in the diets (KJ) | 14,679.90 | 14,650.90 | - | - |
| Apparent fat digestibility,% | 76.67 | 80.35 | 0.938 | 0.040 |
| Apparent GE digestibility,% | 70.58 | 71.34 | 0.729 | 0.648 |
| Energy intake | 10,322.61 | 10,249.77 | - | - |
| Percentage of energy intake that is : | | | | |
| Energy stored in the body | 41.48 | 43.98 | 2.245 | 0.625 |
| Energy expenditure | 29.10 | 27.36 | 2.252 | 0.734 |
| Lost in excreta | 29.42 | 28.66 | 0.729 | 0.648 |

**Results and discussion** Apparent fat digestibility was improved in SBO diet (P=0.040) but gross energy digestibility was no significant different (P=0.648). The SBO diets trended to decrease for energy expenditure and energy in excreta. The energy stored in the body was lower in BT diets than in the SBO fed groups.

Acknowledgements Thanks to Sakon Nakhon Agricultural Research and Traning Center, Rajamangala Institute of Technology for financial and facility supports.

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# Effect of different levels of pigment and fat sources on blood and egg yolk cholesterol and yolk colour of laying hens

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**Introduction** Egg yolk colour has always been regarded as an important egg quality characteristic and recently has had an even more important role in the marketing of eggs. One of the dietary factors influencing egg yolk colour is dietary fat. Dietary fat has been reported to have a positive or negative effect. Mackay et al (1963) reported that the addition of 4% animal fat improve egg yolk pigmentation. Hamilton and Parkhurst (1990) found that fats with lowering melting point resulted in higher oxycarotenoid level in yolk. An investigation of the role for dietary fat in yolk pigmentation appeared worthwhile because the sources of fat are different in the formulation of diets and because yolk pigmentation is an economically important quality factor. The objective of the present study was to investigate the influence of three sources of fat and levels of pigment on yolk colour and performance of laying hens.

**Materials and methods** One hundred and sixty two Hy-line W36 laying hens (23 weeks of ages) were used in this assay. Three basal diets containing either 4% sunflower oil, tallow or fatty acids were prepared. Each of the basal diets was supplemented with three levels of natural pigment (0 and 1100 ppm red pigment (BioCaps, Paprika extraction that contains Capsanthin and Capsorubin)with 125 ppm yellow pigment ( BioGold, Marigold extraction that contains Zeaxanthin), 1500 ppm red pigment with 225 ppm yellow pigment). Egg production, egg weight, feed efficiency; yolk colour, Serum and egg cholesterol was measured. Blood samples were collected from the wing vein of individual birds and sera were harvested for cholesterol determination. at the end of each weekly period, Egg were collected for three consecutive days of each weekly period and a pooled egg yolk sample was used for determination of cholesterol content (Sim and Breagg, 1977). Colour of the yolk was weekly measured by Roch Yolk Colour Fan. Data were subjected to ANOVA procedures appropriate for completely randomized design (CRD) and analyzed as 3*3 factorial arrangements. Multiple range comparison was made according to Duncan to determine significant difference among treatment means.

**Results** The effect of fat sources on blood cholesterol was significant (P<0.05). Tallow showed the highest and fatty acid showed the lowest effect on blood cholesterol (table1). Sim et al (1980) reported that the effect of plant sterols in cholesterol metabolism may be attributed to the inhibition of cholesterol absorption in gut, because the fecal excretion of the sterol metabolites is enhanced when soysterol is fed. The main effect of fat on yolk cholesterol was significant (P<0.05). Sunflower oil had the most and tallow had the leas effect on yolk cholesterol (table1). Lall and Slinger (1973) have shown that the presence of high level of polyunsaturated fats in the diet increase egg yolk cholesterol. Supplementation of pigments to the diets had significant effects on yolk colour. The main effect of fat on yolk colour was significant (P<0.05). Tallow had the highest score than fatty acid and sunflower oil at the end of the trial (table2).

**Table 1** Effect of fat sources on performance and cholesterol concentration of serum and egg yolk in laying hens

| Treatments | Egg production (%) | Yolk weight (gr) | Feed efficiency ratio | Blood cholesterol (mg/dl) | Yolk cholesterol (mg/gr) |
|---|---|---|---|---|---|
| Sunflower oil | 92.58±0.81 | 13.26±0.60 [ab] | 1.90±0.018 | 180.14±13.17[ab] | 6.85±0.27 [a] |
| Tallow | 92.62±0.80 | 13.11±0.70 [b] | 1.89±0.018 | 188.78±13.34 [a] | 5.65±0.27 [b] |
| Fatty acid | 91.16±0.81 | 13.51±0.60 [a] | 1.92±0.019 | 146.46±13.43 [b] | 6.20±0.27 [ab] |

[a-b] Any two means for a factor with no common superscripts are significantly different (P<0.05)

**Table 2** Effect of fat sources on yolk colour score

| Treatments | First week | Second week | fifth week | Last week |
|---|---|---|---|---|
| Sunflower oil | 7.33±0.147 [b] | 8.39±0.091 [b] | 9.06±0.091 | 8.56±0.143[b] |
| Tallow | 8.56±0.147 [a] | 9.06±0.091[a] | 9.11±0.091 | 9.06±0.143 [a] |
| Fatty acid | 8.78±0.147 [a] | 9.17±0.091 [a] | 9.28±0.091 | 8.28±0.143 [b] |

[a-b] Any two means for a factor with no common superscripts are significantly different (P<0.05)

**Conclusions** Using natural pigments in laying hens diets will improve egg yolk colour. Source of the fat will affect yolk colour and weight and also blood and egg cholesterol content. Fats with high level of saturated fatty acids have the most effect on egg yolk colour.

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# Investigation of feeding betaine as an osmoprotectant in broiler chicks

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**Introduction** Trimethylglycine has two primary metabolic roles: it is a methyl group donor and an osmolyte that assists in cellular water homeostasis. Tissues that rely on zwitterionic betaine as an osmolyte include the intestines, kidney, liver, brain and leukocytes. Osmolytes are particularly important in situations in which cellular dehydration is present because these compounds help minimize water loss despite a prevailing osmotic gradient. Thus, water balance homeostasis is an important factor for cells exposed to a variety of osmotic conditions (Klasing et al., 2002). For example, the osmotic pressure of the intestinal contents varies during the process of digestion. The osmotic difference between the intestinal epithelium and the luminal fluid is likely to necessitate means to control the osmotic pressure inside the intestinal epithelial cells. Betaine, as an organic compatible osmolyte, is one of the most likely candidates for the task.

**Materials and methods** 576-day-old male broiler chicks (Ross308) were used in a factorial arrangement of treatments in a CRD experiment with 4 levels of added betaine (0.00, 0.75, 1.50 and 2.25g/kg diet) × 3 levels of water TDS (375, 1375 and 2375mg/l) each in 4 replicates. Levels of TDS were made by adding NaCl to basal drinking water. TDS of the basal water was 375mg/l. Diet provided nutrient requirements for broiler chicks according to Ross308 management manual and the met+cys levels were exactly met the requirement, which eliminates the methionine-sparing effect of betaine. Feed and water consumed *ad libitum*. Prior to begin the trial, two birds of each replicate chosen randomly and wing banded. At 4 and 6 weeks of age water consumption were measured. At the same ages, excreta were collected, subsequently dried in an oven at 65ºC for 72 hours in order to determine excreta moisture. At 28 and 42-d two ml of blood was taken from the brachial vein of wing banded birds and centrifuged immediately. Separated plasma was used for determination of plasma osmolarity, Na, K, Cl and albumin concentration. According to the method of Klasing *et al*. (2002) samples of intestinal epithelia were taken from 2 birds from each replicate for osmolarity evaluation along the intestine. Osmolarity was measured by using a freezing point osmometer. Data were analyzed using the GLM procedure of SAS. Significant difference among individual group means was determined with Duncan's multiple range tests.

**Results** Dietary betaine increased plasma Na concentration at 28-d ($P<0.05$) and decreased PCV at 42-d ($P<0.05$). Betaine had no significant effect on plasma osmolarity, K, Cl, and albumin concentration of plasma at 28-d and 42-d, plasma Na concentration (at 42-d) and also epithelia osmolarity of duodenum and jejunum at 42-d. TDS showed an increasing effect on PCV, osmolarity of duodenum, jejunum and ileum, plasma Cl and albumin concentration at 42-d, but this effect was not significant. Water consumption of 4 and 6 weeks of age and excreta moisture of fourth weeks of age were increased from the first to third level of salinity ($P<0.01$). Epithelia osmolarity was decreased from duodenum to ileum, these results are in agreement to those reported by Klasing and co-workers (2002). Our observed value of 760 mOsmol in the duodenum is very hyperosmotic compared with normal plasma, which we found to be 330 mOsmol. Birds consumed feed *ad libitum* and the intestines were full of digesta; thus, the high osmolarity could be the result of active absorption of nutrients. Furthermore, interaction effect was significant for plasma osmolarity at 28-d and epithelia osmolarity of duodenum ($P<0.05$).

**Table 1** Evaluation the osmolytic effect of betaine in broiler chicks, fed with dietary betaine and saline water †

| Main effect | Water consumption | | Excreta moisture | | PCV | Osmolarity | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Plasma | | Intestinal epithelia | | |
| | 4week | 6week | 4week | 6week | 42-d | 28-d | 42-d | Duodenum | Jejunum | Ileum |
| | --(ml : bird : day)-- | | ------(%)------ | | (%) | ----------(mOsmol)---------- | | | | |
| TDS | | | | | | | | | | |
| 375 | 227[b] | 385[c] | 80[b] | 81 | 29.5 | 304 | 331 | 705 | 687 | 540 |
| 1375 | 266[a] | 410[b] | 82[a] | 81 | 30.4 | 304 | 329 | 727 | 711 | 584 |
| 2375 | 282[a] | 464[a] | 83[a] | 83 | 32.3 | 305 | 331 | 849 | 799 | 656 |
| SEM | 5.72 | 7.38 | 0.65 | 0.54 | 0.96 | 1.31 | 2.14 | 46.19 | 46.64 | 50.13 |
| Betaine | | | | | | | | | | |
| 0.00 | 248 | 405 | 82 | 83 | 34.4[a] | 305 | 332 | 649 | 660 | 486 |
| 0.75 | 261 | 416 | 81 | 82 | 30.1[b] | 304 | 327 | 780 | 781 | 578 |
| 1.50 | 264 | 432 | 82 | 81 | 29.0[b] | 305 | 332 | 848 | 748 | 626 |
| 2.25 | 260 | 426 | 82 | 81 | 29.2[b] | 303 | 331 | 764 | 740 | 687 |
| SEM | 6.6 | 8.52 | 0.75 | 0.62 | 1.08 | 1.51 | 2.47 | 53.33 | 53.86 | 57.88 |

† [a, b,c] Means in a column with different superscripts differ significantly($P< 0.01$)

**Conclusion** It seems that body homeostatic regulation system defend against changes of blood parameters and increased total volume of blood. The non significant increasing effect of TDS on percentage of PCV indicates that salt tolerance is also affected by species, strain, and individual variation.

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# Effect of water quality on *in vitro* fermentation of sorghum and barley for poultry diets

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## Introduction

Low pH and high lactic acid concentration of fermented feed has been reported to be responsible for the antimicrobial activity of fermented feeds (Brooks *et al.*, 2001). For example, to prevent the growth of *Salmonella* spp. in liquid feeds, a threshold lactic acid concentration of 75mM is required (Beal *et al*., 2002). Therefore, factors that are likely to affect the production of lactic acid during fermentation will have important implications for the ability of such feeds to withstand colonisation by pathogens. The objective of the present study was to investigate the effect of water quality on the fermentation pattern of sorghum and barley.

**Materials and methods** The study was conducted as a three factor, factorial design. Factor 1 was grain type (sorghum or barley), Factor 2 was lactic acid bacteria (LAB) inoculant (*Lactobacillus farciminis* (F)*, L. plantarum* (S) *or Pedococcus acidilacti* (P)) and Factor 3 was water mineral content (five concentrations of calcium carbonate in distilled water $T_0$ ($0gl^{-1}$), $T_{25}$ ($0.01gl^{-1}$), $T_{50}$ ($0.02gl^{-1}$), $T_{75}$ ($0.03gl^{-1}$) or $T_{100}$ ($0.04gl^{-1}$). All cereal samples were hammer milled and irradiated (25 kGy from $Co^{60}$) in 100 g sachets before fermentation. There were three replicates per treatment. Feed samples were mixed with water at a ratio of 1:1.4; inoculated with 0.1 ml Mann rogosa Sharp (MRS) broth containing one of three LAB (providing a concentration of ca $10^6$ cfu $g^{-1}$ of feed) and incubated at 30 ºC. The pH was recorded after 24 hour fermentation and samples were taken for analysis of lactic and acetic acids by HPLC according to the method of Niven *et al.*(2004). All data were analysed by analysis of variance using Minitab (release 14.0).

**Results** The correlation between total acid content and pH for barley (Figure 1) was slightly positive ($R^2$=11.76), with pH increasing slightly as lactic acid concentration increased. Overall there were no significant differences in post fermentation pH between the grains. However, highly significant differences (P<0.001) in pH were observed for LAB and water mineral content and for the interaction between them. The lowest overall pH value of 3.37 (SEM=0.0213) was recorded from sorghum fermented with *L. plantarum* in water containing 0.04 $gl^{-1}$ $CaCO_3$ ($T_{100}$). Meanwhile, the only pH value which was above 4 for the whole experiment was obtained with in sorghum fermented with *L. farciminis* in water containing 0.02 g $l^{-1}$ $CaCO_3$ ($T_{50}$). The variation in lactic acid concentration was highly significant (P<0.001) for all the factors and their interaction. Overall, lactic acid production was significantly higher (P<0.001) in barley, with an overall mean of 405 mM (SEM=6.41) compared with 286 mM (SEM=6.29) for sorghum (Figure 2). With barley, the production of lactic acid did not show any particular pattern for any of the organisms. The pattern was more regular for sorghum, with water containing no carbonate ($T_0$) giving a significantly higher (P<0.001) lactic acid content than water containing $CaCO_3$ regardless of its concentration. The effect of water quality and its interaction with grain and LAB was not significant for the acetic acid content, which remained below 50 mM (SEM=6.03) for all fermentations.

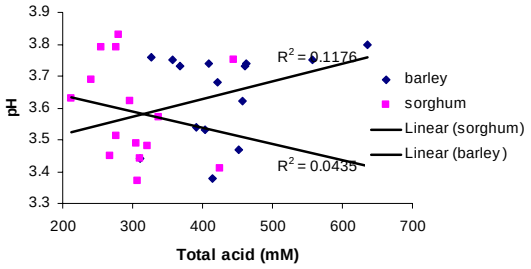


**Figure 1: Relationship between pH and total acid.**

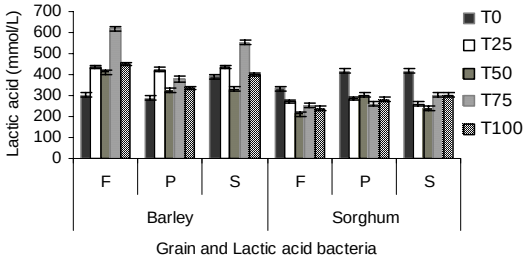


**Figure 2:** Effect of water quality on lactic acid fermentation of barley and sorghum

**Conclusion** From the data presented here, it is apparent that the calcium content of the water has an effect on lactic acid production from barley and sorghum. Calcium appeared to inhibit lactic acid production in sorghum whereas in barley the relationship is not clear. The resistance to a drop in pH with increasing lactic acid concentration in fermented barley indicates calcium may affect the buffering capacity of the grain. Therefore, pH of fermented cereals might not be good indicator of the lactic acid content.

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# Precaecal crude and amino acid digestibility in the seeds of a tropical crop (*Manihot esculenta*) in broilers

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**Introduction** There is a growing interest in the use of lesser known plant seeds as sources of protein in monogastric feeding in the tropics. It is thought that the exploitation of novel seeds which are currently not utilized by man can contribute to the amelioration of the insufficient feed supply situation in tropical countries like Nigeria. The seeds of *Manihot esculenta* (Cassava) have the potential to complement the conventional plant protein in poultry diets. Lack of information on the nutritional quality and digestibility of the protein and amino acids in the seeds has caused a limitation in their use for poultry feeding. The objectives of this study were 1) to investigate the chemical and antinutritional constituents in the seeds of *Manihot esculenta*, and 2) to determine the precaecal digestibility of crude protein (CP) and amino acids (AAs) in the seeds with broilers and their effect on the performance of the birds.

**Materials and methods** Seeds of *M. esculenta* were sun dried to a constant weight and ground to pass through a 2 mm sieve. The seed meal (MASM) was incorporated at levels of 75 and 150 g/kg in place of corn starch in broiler diets. Titanium dioxide ($TiO_2$) was included as an indigestible marker at a level of 5 g/kg. One hundred and eighty male 1-d-old broiler chicks (Ross strain) were allocated to 18 pens of 1.7 $m^2$ in groups of 10 birds each in an illuminated and temperature-controlled room. They were fed a commercial diet for 14 days and thereafter the test diets for a further 7days. Feed and water were supplied *ad libitum*. AA analysis in the seeds, diets and digesta was by the method of Naumann and Bassler (1976). The concentration of $TiO_2$ in the diets and digesta were determined according to the method of Brandt and Allam (1987). The precaecal digestibility coefficients of the CP and AAs for each diet were calculated according to the following equation:

$$DC_{AADiet} = 1-[(TiO_{2Diet} \times AA_{Digesta}) / (TiO_{2Digesta} \times AA_{Diet})]$$

The precaecal digestibility of AAs in MASM was calculated according to the following equation:

$$D_{Ti} = D_{TD} - D_{BD} \times (1-A_{Ti}) / A_{Ti}$$

Data were analysed using the ANOVA (SPSS 11.0 for Windows) (SPSS Inc., Chicago IL, USA).

**Results** MASM in the diets resulted in higher values of digestibility of AAs (Table 1). At 150 g $kg^{-1}$ level of inclusion of MASM, digestibility of CP and feed intake of the birds were significantly ($P<0.05$) higher than those of birds on the basal diet (Table 2). Birds on the MASM diets had significantly higher weights than those on the basal diet. The higher digestibility of CP and AAs in the birds on the MASM diets resulted in a better performance in the birds. This is an indication that birds may well tolerate levels of 150 g $kg^{-1}$ of MASM in their diets

**Table 1** Precaecal CP and AA digestibility in experimental diets (n = 6 pens of 10 birds per treatment)

| | Basal | MASM | | | |
|---|---|---|---|---|---|
| Level of seed inclusion (g $kg^{-1}$) | - | 75 | 150 | SEM | *P* (ANOVA) |
| Crude protein | 0.71[a] | 0.68[a] | 0.74[b] | 0.05 | <0.01 |
| Arginine | 0.67[a] | 0.68[a] | 0.77[b] | 0.04 | 0.018 |
| Isoleucine | 0.73[a] | 0.76[e] | 0.77[a] | 0.06 | 0.001 |
| Leucine | 0.78[a] | 0.83[b] | 0.84[b] | 0.05 | <0.001 |
| Lysine | 0.73[a] | 0.71[a] | 0.74[a] | 0.06 | 0.003 |
| Methionine | 0.76[a] | 0.76[a] | 0.81[b] | 0.05 | <0.001 |
| Phenylalanine | 0.79[a] | 0.85[b] | 0.85[b] | 0.05 | <0.001 |
| Threonine | 0.61[a] | 0.74[b] | 0.74[b] | 0.05 | <0.001 |
| Valine | 0.72[a] | 0.78[b] | 0.82[b] | 0.07 | 0.001 |

[a,b,c,d,e,f,g] Values with different letters on same row differ significantly ($P<0.05$) according to LSD

**Table 2** Feed intake and weights of birds on experimental diets (n=6 pens of 10 birds per treatment)

| | Basal | MASM | | | |
|---|---|---|---|---|---|
| Level of seed inclusion (g/$kg^{-1}$) | - | 75 | 150 | SEM | P value |
| Total feed intake (g) | 395[a] | 459[b] | 480[b] | 12.9 | <0.01 |
| Final weight (g) | 691[a] | 782[b] | 788[b] | 27.1 | <0.01 |

[a,b,c,d,e,f] Values on same row with different letters differ significantly ($P<0.05$) according to LSD

**Conclusion** Results of the study have shown that the digestibility of CP and AAs was increased in diets containing the seed meal with a resultant improvement in performance of the birds. Thus seeds of *M. esculenta* can serve as a source of plant protein in diets of broilers.

**Acknowledgement** The financial assistance of the Alexander von Humboldt Foundation for this study is gratefully acknowledged.

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# Study on the effects of *Saccharomyces cerevisiae SC47* on broiler performance and immune organs

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**Introduction** Several researches was conducted to investigate the effect of *Saccharomyces cerevisiae* on poultry performance, but *Saccharomyces cerevisiae* has an especial quality that makes its more noticeable for continuing research about its. This quality is:a) improving fatty acid digestibility( Kautz and Arens,1998), Improving of ether extract, cellulose digestibility and retention of nitrogen 2) improving of Phosphors digestibility(3) b) Stimulation of immunity c)improving of bronchitis and gambro Elisa titer d) have a several strain.According to this, an exprement was conducted to investigated the effects of SC47 on broilers performance and immune organs.

**Material and methods** These experiments consist of 5 treatments (0 , 0.25, 0.5, 0.75 and 1 g *SC* per Kg of Diet) and 4 replicate . 400 day- old chicks were randomly assigned to 20 litter floor pens..In experimental period (1-42 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 then carcass quality ( carcass weigh (%), breast, leg, abdominal fat, liver and gizzard ) and immune organs (Borsa, spleen and timos) weight were measured. After measuring Immune organs (Borsa, spleen and timos ) weight , the percentage of them to live body weight were calculated.

**Result** The result showed that feed intake, weight gain, feed conversion and production index in yeast group improved but the differences were not significant.($p<0.05$). Using yeast in group 2,3,4 and 5 improved feed intake in comparison to control by 1.21, 5.14, 6.14 and 3.16 and also weight gain increased by 5.93, 5.93, 3.45 and 1.2 (%) respectively. Using yeast (*SC*) in broilers diet decreased the percentage of mortality ($p<0.05$). The percentage of mortality was 3.5, 1.75, 1, 1.25 and 3.75 (%) in groups 1, 2, 3, 4 and 5 respectively. Mortality in group 5 was lower than controls but higher than other yeast group .

Effect of of yeast (*Saccharomyces cerevisiae SC47 )* utilization on the commercial broiler performance

| Age (day) | Variable | Yeast levels(%) | | | | | SE |
|---|---|---|---|---|---|---|---|
| | | 0 | .025 | .05 | .075 | .1 | |
| 0-42 | Feed Intake (g/h/d) | 85.80 | 88.86 | 90.21 | 91.07 | 88.51 | 0.95 |
| | Weight gain ( g/d) | 40.82 | 43.24 | 43.26 | 42.23 | 41.31 | 0.51 |
| | Feed conversion Ratio | 2.101 | 2.057 | 2.088 | 2.155 | 2.146 | 0.02 |
| | Mortality (%) | 3.50 a | 1.75 abc | 1 c | 1.25 bc | 2.75 ab | |
| | Production Index | 203.05 | 216.01 | 213.01 | 202.97 | 199.85 | 3.29 |
| | Timous glands (%) | 0.320 | 0.373 | 0.329 | 0.371 | 0.321 | 0.017 |
| | Spleen (%) | 0.103 | 0.105 | 0.114 | 0.117 | 0.103 | 0.004 |
| | Bursal gland (%) | 0.138 | 0.162 | 0.151 | 0.144 | 0.149 | 0.010 |

[a-c]Mean within a row followed by different superscripts statistically different($p\leq.05$)

The result showed that using yeast (S.C) had no positive effect on carcass, leg, breast and abdominal fats, liver, heart, gizzard and immune organs ($p<0.05$).

**Discussion** Using yeast in broiler diet improved feed intake, weight gain, feed conversion and production index but the differences were not significant . This result were agree with Durst and et al,(1995) that they reported that using *SC* in broilers diet improve average daily weight gain but differences were not significant. Feed conversion efficacy , weight of the carcass, stomach and intestine were not affected by using *SC*.
Immune organs in yeast group were higher than control, and its maybe because of the stimulation of yeast. Generally these properties are related to the presence, in the inner part of yeast cell wall, of glucans, that are constituted of main chains of beta-(1-3)-linked D-Glucose molecules to which are attached linear side chain of beta-(1-6)linked residues. These macromolecules, have an ability to stimulate certain aspects of the immune system in mammal's especially inflammatory response and reticuloenthelial system. Increase in weight and size of the reticuloenthelial system (liver, spleen and lungs) was also observed following glucan treatment ( 2)

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## Growth-promoters utilization in diet for broilers chicken

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**Introduction** In the productive chain of broiler chicken the sanitary problems are minimized with the use of alimentary additives. The official Brazilian Health Department has established criteria for the use of antibiotic in broilers chicken diet and the use of probiotics, prebiotics and simbiotics has increased (Miltenburg, 2000). This paper was developed to evaluate the effect of different growth-promoters in diet for broilers chickens from 1 to 21 days age on weight gain, feed intake and feed conversion and to study the economic viability of addition of those promoters.

**Material and methods** Four hundred eighty Ross broilers chicken were assigned to a randomised blocks design totalising five treatments and four replications, with 24 birds in each one. Treatments consisted of: T1-control; T2-control plus antibiotic (colistin and Zn-Bacitracin); T3-control plus probiotic (Protexin);T4-control plus prebiotic (Bio-mos); T5-control plus probiotic and prebiotic. The experimental diets were formulated for to attempt the nutritional requirements according to Rostagno et al. (2005). The light program was continuous during the 24 hours of the day, being during the day with natural illumination, and the night with artificial light, using fluorescent light bulbs of 100 watts. The variables feed intake, weight gain, feed conversion of the birds in the period from 1 to 21 days of age and the economic viability of the use of different growth-promoters were evaluated. The average values of temperature and humidity in the experimental environment were, respectively, of 30.58±2.02°C and 56.27±5.27%. Data of the performance variables were analysed by ANOVA and Student-Newman – Keuls (SNK). The study of economic viability was according to Ramos et al (2005).

**Results and discussion** There was no difference among treatment (P>0.05) for the parameters feed intake, weight gain and feed conversion (Table 1). However, in terms of absolute values when the average of the weight gain (g) of the treatments is compared, it was observed that the T5 (probiotic and prebiotic) was superior to the T1 (control) and T2 (antibiotic), respectively, with the percentile value of 9.72% and 4.41%. This fact shows that the association of probiotioc and prebiotic (symbiotic) are important alternatives as a substitute of antibiotics in broiler chickens diets. The economic evaluation showed an average gross profit margin of 6.0% superior for diets with symbiotic in relation to diet with antibiotic. The ration control and with prebiotic presented minor economic yield.

**Table 1** Performance and economic index for broilers chickens fed ration with growth promoter from 1-21 days age.

| Variables | Treatment | | | | | |
|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | T5 | VC (%) |
| Feed intake | 1119.00[a] | 1115.75[a] | 1129.00[a] | 1141.50[a] | 1067.25[a] | 6.31 |
| Weight gain | 774.33[a] | 824.00[a] | 813.75[a] | 781.75[a] | 820.25[a] | 2.96 |
| Feed conversion | 1.44[a] | 1.36[a] | 1.39[a] | 1.46[a] | 1.30[a] | 8.31 |
| Average feeding cost | 0.63 | 0.66 | 0.63 | 0.61 | 0.64 | -- |
| Average feeding cost: weight gain ratio | 0.91 | 0.89 | 0.87 | 0.89 | 0.83 | -- |
| Gross profit | 1.48 | 1.57 | 1.55 | 1.49 | 1.56 | -- |
| Average gross profit margin | 0.78 | 0.83 | 0.84 | 0.79 | 0.88 | -- |

[a] Means followed by same letter in line are not different (P<0.05)

**Conclusions** The findings of this study suggest that the association of probiotic and prebiotic (symbiotic) is an alternative to poultry industry to substitute the antibiotic in the diets. The use of probiotic and prebiotic is conditioned to the price of ration.

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# Performance and carcass characteristics of broiler chickens fed diets supplemented with graded levels of feed enzyme Roxazyme-G®

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**Introduction** Feed remains the most important cost in animal production. The need for feed ingredients, which will reduce the cost of production, is the basis for most livestock feed and production research. Man and his livestock are in competition for the basic ingredients. Availability of such feed ingredients thus becomes the key limiting factor in poultry production, hence the need for alternative and locally available feedstuffs. The presence of undegradable and complex carbohydrate, non-starch polysaccharides (NSPs) in some alternative and locally available feedstuffs exerts some anti-nutritional properties and thus can limit their use in livestock feeding (Yoruk *et al.,* 2006). Worse still is the fact that chickens are not capable of hydrolyzing NSPs that mask protein carbohydrate (Petterson and Aman, 1989). Exogenous enzyme supplementation has been reported to be used in poultry diets to improve nutrient utilization, the health and welfare of the birds, product quality and to reduce pollution as well as to increase the choice and content of ingredients which are acceptable for inclusion in diets (Aderemi *et al*., 2006). Since most work with exogenous enzymes has been carried out to evaluate its effect on feed intake and growth characteristics of broilers, the aim of this study was to investigate the effect of graded level of feed enzyme Roxazyme-G® on the performance, carcass and meat qualities of broiler finishers.

**Materials and methods** A total of three hundred and sixty (360) unsexed three week-old Anak strain broiler chicken were randomly allocated to four experimental dietary treatment groups in a completely randomized design with each dietary treatment group replicated thrice to evaluate the effect of Roxazyme-G® (Cellulase endo-1, 4-β-glucanase; endo-1, 3(4) - β-glucanase; endo-1, 4- β-xylanase) on the performance and carcass characteristics of broiler chickens. The birds were fed the experimental diets containing 0, 0.1, 0.2 and 0.3% enzyme supplementation for a 35-day period during which data were obtained on feed intake, weight gain, dry matter digestibility (DMD) and feed conversion ratio. At the end of the feeding trial, ten birds were sacrificed per replicate to evaluate the carcass and meat characteristics of the birds. All data obtained were subjected to analysis of variance and where statistical significance was observed, the means were compared using the Duncan Multiple Range (DMR) test. The SAS Computer software package was used for all statistical analysis (SAS, 1998).

**Results** The inclusion of the exogenous enzyme did not ($P>0.05$) improve the average weight gain, feed intake, feed conversion ratio and DMD. The dressing percentage of birds fed the enzyme-supplemented diets was significantly ($P<0.05$) superior compared to the control. There was no effect of treatment ($P>0.05$) on any of the primal cuts but the head and neck of the birds on the control diet were lower ($P<0.05$) in weight compared with the other treatments. The inclusion of the enzyme did not ($P>0.05$) affect the relative weights of the kidney, gizzard, heart and the liver. The flavour, tenderness and juiciness scores of the meat of birds fed the enzyme supplemented diets were higher ($P<0.05$) than the control while the colour, texture and the overall acceptability were not affected ($P>0.05$) by the inclusion of the enzyme in the diet. The Waner Braztler shear force result showed no increase ($P>0.05$) in toughness in agreement with the sensory panel result that judged the meat from birds fed enzyme supplemented diets as more tender ($P<0.05$) than that of the control. The breast muscle of the chickens had higher cooking loss than the thigh muscle while the highest ($P<0.05$) cooking loss was recorded for both muscle parts of birds reared on 0.2% enzyme supplementation.

**Table 1**: Performance and carcass cuts of broiler finisher fed graded levels of feed enzyme (Roxazyme-G®)

| Parameters | Inclusion level of feed enzyme (Roxazyme-G®) | | | |
|---|---|---|---|---|
| | 0.00 | 0.10 | 0.20 | 0.30 |
| Average feed intake (g/day) | 101.13±7.28 | 102.15±7.04 | 103.39±6.31 | 101.38±7.33 |
| Average weight gain (g/day) | 18.79±2.22 | 20.97±2.34 | 20.77±2.41 | 19.04±2.15 |
| Dry matter digestibility (%) | 67.61±3.15 | 71.03±4.01 | 69.20±1.89 | 67.55±4.11 |
| Feed conversion ratio | 2.67±0.81 | 2.57±0.09 | 2.65±0.11 | 2.77±0.14 |
| Dressing (%) | 72.83±1.76$^{b}$ | 75.70±0.96$^{a}$ | 77.82±0.98$^{a}$ | 75.97±4.54$^{a}$ |
| Head (%) | 2.41±0.03$^{b}$ | 2.74±0.08$^{a}$ | 2.87±0.05$^{a}$ | 2.64±0.10$^{ab}$ |
| Neck (%) | 4.65±0.12$^{b}$ | 5.84±0.08$^{a}$ | 5.55±0.15$^{a}$ | 5.10±0.11$^{ab}$ |

a,b=Means along the same row with similar superscripts are not significantly ($P>0.05$) different from each other.

**Conclusion** Overall, although the effect of the feed enzyme was not pronounced on the performance characteristics, from the results of the carcass characteristics of the broiler chickens, it could be concluded that supplementing the diets of broiler finisher birds with Roxazyme-G® increased the dressing percentage and enhanced the meat quality in terms of flavour, tenderness and juiciness.

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# Effect of broiler breeder age and glutamine on the development of the intestinal mucosa of chicks during the first week of the age

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**Introduction** The effect of glutamine on the structure of the intestinal mucosa, after suffering any damage is raising interest due to the fact that this amino acid is the main metabolite which nourishes the enterocytes. Yet, the mechanism that promotes proliferation of the intestinal cells is not well known. It might be related with two events: increase of $Na^+/H^{+-}$ exchange at the plasmatic membrane and increase of ornithine descarboxylase specific activity. Glutamine is also able to raise gene transcription, by increasing the activity of kinase protein which activates mitogenesis (Blikslarge *et* al., 1997). Chick's weight is determinated by eggs initial weight. Older breeders produce bigger eggs which become chicks with higher weight at eclosion moment. In other words, higher consumption levels of protein at the beginning of laying, provide better albumen quality which becomes thicker, delaying oxygen exchanges, the absorption of yolk sac and embryo uptake of vitamins through egg yolk (Brake, 1995). As the embryo produces 90% of its energy from fat acid oxidative process, oxygen deficit would slow lipid oxidation and embryo development. The purpose of this study was to evaluate the development of the intestinal mucosa of chicks at 7 days of age, proceeding from breeders of different ages and fed with and without glutamine supplement.

**Material and methods** Thirty-two Cobb-500$^{TM}$ male chicks were used. Immediately after hatching, chicks were housed in an environmentally controlled room where ambient temperature was maintained at thermo neutrality (33$^o$ C) with continuous light. Chicks were distributed in battery pens with 8 chicks each divided into 4 groups: Chicks from broiler breeder with 30 weeks of age with glutamine supplement; chicks from broiler breeder with 60 weeks of age with glutamine supplement; chicks from broiler breeder with 30 weeks of age without glutamine supplement; and chicks from broiler breeder with 60 weeks of age without glutamine supplement. Water and feed were *ad libitum*. Seven days after hatching, 8 birds per treatment were slaughtered by cervical dislocation and, small intestine were measured (cm ± 0.01) and collected for analysis under light microscope, scanning and transmission electron microscope.

**Results** Broiler breeder age showed no effect on the studied variables. At eclosion moment, embryos from older breeders presented a more developed intestinal mucosa, when compared to embryos from younger breeders. However this fact did not maintained at the end of the pre-initial stage. Glutamine supplement had a positive effect on villous height at duodenum (P= 0.009), jejunum (P= 0.006) and ileum (P= 0.001), cryptal depth at jejunum (P= 0.037). Glutamine is known to be an energetic substratum vital to rapid division cells, such as intestinal cells, stimulating its proliferation Broiler chicken forced to quantitative feeding restrictions and supplemented with L-glutamine present higher villous due to the fact that this birds also present higher expression of ornithine decarboxylase enzyme. Glutamine could have an important role on the maturation of chick intestinal mucosa during the first week of age.

**Table 1** Villous height (μm) and crypt depth (μm) at duodenum, jejunum and ileum.

| Broiler breeder age (weeks) | Glut | Duodenum (μm) | | Jejunum (μm) | | Ileum (μm) | |
|---|---|---|---|---|---|---|---|
| | | Villous | Crypt | Villous | Crypt | Villous | Crypt |
| 30 | (+) | 1131±39 | 170±26 | 595±70 | 96±26 | 505±31 | 91±12 |
| 30 | (-) | 1076±28 | 168±34 | 535±87 | 85±20 | 450±42 | 92±13 |
| 60 | (+) | 1119±57 | 171±28 | 598±71 | 96±24 | 508±30 | 89±14 |
| 60 | (-) | 1103±71 | 165±36 | 531±85 | 85±19 | 449±36 | 90±13 |
| | | Main effects | | | | | |
| Broiler breeder age (weeks) | 30 | 1104 | 168 | 566 | 90 | 476 | 90 |
| | 60 | 1111 | 169 | 565 | 91 | 479 | 90 |
| Glutamine | (+) | 1125 | 170 | 597 | 95 | 506 | 91 |
| | (-) | 1090 | 167 | 534 | 85 | 449 | 90 |
| | | Probability | | | | | |
| Broiler breeder age (A) | | 0.573 | 0.852 | 0.966 | 0.952 | 0.869 | 0.518 |
| Glutamine (B) | | 0.009 | 0.644 | 0.006 | 0.037 | 0.001 | 0.892 |
| AxB | | 0.127 | 0.756 | 0.841 | 0.920 | 0.830 | 0.957 |

**Conclusions** Broiler breeder age revealed no effect on the development of the intestinal mucosa after the first week of life; Inclusion of glutamine seems to have a positive effect of the development of duodenum, jejunum and ileum villous, during broiler chick's first week of life.

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# Four statistical tests for detecting major genes in an Iranian pedigree chicken flock

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**Introduction** Identification of single genes with large effect (major genes) on quantitative traits of economic importance is essential prior to applying gene and marker assisted selection. It is well known that if a major gene of large effect segregates in a population, the distribution of the trait would deviate from normal distribution. Also, heterogeneity of variances across full-sib and half-sib families can be expected. As well, a significant relationship between the family mean and family variance when the effect of a major gene exists is created. There are several ways for detecting major genes using various simple and elaborate methods. Leroy and Elsen (1992) proved that the most powerful tests are the within family-variance heterogeneity test (Bartlett test) and the within-family mean-variance regression (Fain test).
The objective of this research was to look for the evidence of segregating major genes in an Iranian pedigree chicken flock (Mazandaran station), using four statistical tests: Normality, Bartlett, Fain and Mixture tests for four economically important traits. These traits are age at first egg (AFE), body weight at the end of week eight (BW8), average egg weight (EW) and number of eggs laid during egg production period (EN).

**Materials and methods** Data used in this research were collected from a pedigree indigenous chicken flock located in North of Iran (Mazandaran province) through 14 generations of selection. Selection in this population had been based on a selection index during the first few generations followed by selection using estimated breeding values produced by multiple trait BLUP evaluation. After editing the data, depending on trait, residual values were estimated by an appropriate model. For AFE and EW traits the model included the fixed effects of generation, hatch number and interaction between them (with 58 levels as combined code). For BW8, the sex effect was added to the list of fixed effects. As to EN, before doing statistical analysis, the Box-Cox transformation was examined. Using maximum likelihood method, the value of transformation parameter, Lambda, which maximizes likelihood function, was one (with maximum log likelihood equal to -93155). The value of one for Lambda meant that no transformation was necessary. In the model for analysis of EN the trait length of production period was included in the model as a covariate. In the Bartlett and Fain test half-sib families that had more than 10 offspring were considered. The Mixture test (cluster analysis) was based on fitting a mixture of normal distributions with EM algorithm and Bayesian Information Criterion (BIC).

**Results** In figure 1 phenotypic means for different generations are shown for three traits. This figure shows improvement during 14 generations of selection, especially for BW8 and AFE traits. For all traits statistics of four statistical tests were significant (P<0.01), which indicated the prior evidence of segregating major genes in this population .In the normality test all four criteria (SAS version 9) were significant (P<0.01).In Fain test linear (b1) and quadratic (b2) regressions of half-sib family variance on mean were significantly different from zero for all traits (this was also true for half-sib families with > 100 offspring). In the mixture test residual values had minimum BIC (among 1, 2 and three normal mixture distributions) values with three normal distributions. Proportions of this mixture distribution are shown in table 1(cluster means increase form left to right).

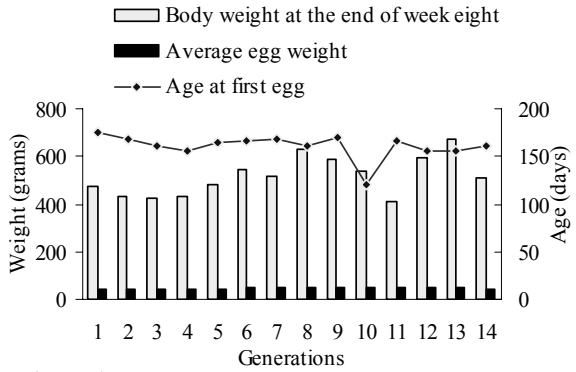


**Figure 1** Phenotypic mean values for three economically important traits in different generations

**Table 1** Mixture test (cluster analysis) results for four traits

| Trait | No. of animals | Min. BIC | Proportions |
|---|---|---|---|
| Age at first egg | 35771 | 286515 | 0.45 0.55 0.00 |
| Average egg weight | 35665 | 194184 | 0.42 0.45 0.13 |
| Body weight | 49157 | 569233 | 0.43 0.20 0.37 |
| Egg number | 36087 | 284886 | 0.28 0.38 0.34 |

**Conclusions** These statistical tests indicate that in this population there are segregating major genes that influence the traits of interest. However considering sensitivity and limited power of these tests, for verification of these results, applying other powerful methods such as Bayesian segregation and linkage analysis is necessary.

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# Performance response and carcass traits of broiler chicks which drank saline water and fed on diets supplemented with betaine

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**Introduction** High levels of TDS in drinking water, has negative effects on broiler performance. Consumption of saline water results in increases blood pressure. In order to expulsion salt, water consumption increases, total amount of blood K decreases and an increment occurs in litter moisture. Consequently performance decreases, anion-cation becomes imbalance and some diseases such as ascites and coccidiosis happen. Change in anion-cation balance affects a lot of physiologic and metabolic functions of body, so can reduces performance and also increases FCR. Nowadays poultry producers in regions with high levels of NaCl in water, try to solve the problem by reducing NaCl content diet, purifying water or etc. Current study examined betaine supplementation for searching another way to get rid of this problem. Betaine implicated in methionine sparing and osmotic stress protection. It can protect intracellular proteins against hyper tonicity induced denaturation. Betaine is a naturally-occurring product present in relatively large quantities in sugar beet and aquatic invertebrates, but is not present in most animal feedstuffs (Wang *et al.* 2004). As a by-product of sugar beet processing, betaine is commercially available as a feed additive (Eklund *et al.* 2005).To date, concerning to our knowledge no information has been published about the osmolytic effects of betaine on performance and carcass traits of broilers in response to saline water consumption.

**Materials and methods** Commercial male broiler chicks were fed diet contains 4 levels of dietary added betaine (0.00, 0.75, 1.50 and 2.25 g/kg) × 3 levels of TDS in drinking water (375, 1375 and 2375 mg/l) as a factorial arrangement from 0 to 42-d. NaCl was added to underground water source in order to made different levels of TDS. TDS of the basal water was 375mg/l. There were 4 replicates of 12 chicks/floor pen. In order to avoid the effects of betaine as a methyl-group donor, sufficient amounts of methyl-donating compounds were added. The diet based on corn soybean meal and contained 5.4, 5.4 and 4.5g methionine/kg for 0 to 10-d, 11 to 28-d and 29 to 42-d, respectively, and 2.7, 2.5 and 2.4g cystine/kg for same periods. Choline chloride (60% choline chloride) which was used for 0 to 10-d, 11 to 28-d and 29 to 42-d was 1.8, 1.6 and 1.4 g/kg. All diets for each period were prepared with the same batch of ingredients, and all diets within a period had the same composition except for the supplemental betaine (Betafin $S_1$, 960 g/kg Betaine). Betaine was added to basal diet from 11-d when the main experiment started. Feed were provided *ad libitum* and water was in free access. At the end of experiment, carcass, breast, abdominal fat, sartorial, liver and heart were weighted. Data were analyzed using GLM procedure of SAS appropriate for CRD experiment.

**Results** Data showed a significant increase in body weight in all ages with increasing dietary betaine and TDS level of water ($P<0.05$). Betaine numerically increased percentages of breast, liver and abdominal fat. Feed intake increased significantly by consuming high levels of TDS at 11 to 21 and 29 to 42-d ($P<0.01$). Increasing the level of water TDS improved FCR of all three periods ($P<0.01$). Chicks which consumed higher levels of TDS showed upper percentages of breast ($P<0.05$). The percentages of sartorial and abdominal fat were decreased in chicks consumed water content 1375 and 2375mg/l TDS ($P>0.05$). Interaction between betaine and TDS was significant on body weight and FCR at 21-d. Cubic relationship was observed between breast yield and TDS at 42-d.

**Table 1** Effect of dietary betaine and TDS of water on performance and carcass yield of broiler chicks at 42-d †

| Main effect | Body weight | FCR | Abdominal fat | Liver | Breast | Carcass |
|---|---|---|---|---|---|---|
| | (42-d) g | (g : g) | ----------(%BW)---------- | | | |
| TDS(mg :l) | | | | | | |
| 375 | 2117[b] | 1.86[a] | 1.63 | 1.98 | 23.99[b] | 73.30 |
| 1375 | 2363[a] | 1.76[b] | 1.57 | 2.01 | 25.45[a] | 72.89 |
| 2375 | 2404[a] | 1.76[b] | 1.56 | 1.95 | 25.60[a] | 74.10 |
| SEM | 24.37 | 0.01 | 0.08 | 0.05 | 0.29 | 0.39 |
| Betaine (g:kg) | | | | | | |
| 0.00 | 2226[b] | 1.86[a] | 1.51 | 1.90 | 24.61 | 73.28 |
| 0.75 | 2292[ab] | 1.78[b] | 1.58 | 1.98 | 24.97 | 73.71 |
| 1.50 | 2320[a] | 1.76[b] | 1.61 | 1.99 | 25.04 | 72.85 |
| 2.25 | 2340[a] | 1.77[b] | 1.65 | 2.05 | 25.43 | 73.89 |
| SEM | 28.14 | 0.02 | 0.09 | 0.05 | 0.34 | 0.46 |

† [a, b] Means in a column with different superscripts differ significantly, $P< 0.05$

**Conclusion** Results showed that betaine is effective in promoting body weight in male broiler chicks. It seems that added betaine may improved digestion and absorption conditions of the gastrointestinal tract and amended the usage of nutrients. This observation corresponded with results obtained in the case of feed efficiency. It shows that betaine maybe involved in the protection of intestinal epithelium against osmotic disturbances which can be caused by different levels of TDS. Moreover, TDS was more effective in decreasing abdominal fat than betaine.

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# The UK National Action Plan on Farm Animal Genetic Resources

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The UK's Farm Animal Genetic Resources (FAnGR) – its farm animal breeds, strains and varieties, and the variability within them - are of great economic, social and cultural importance. For these reasons alone it is important that we care for them, but we also have national and international obligations to do so.

The UK National Action Plan on Farm Animal Genetic Resources was published in November 2006 (see http://www.defra.gov.uk/farm/livestock/strategy/genetic-res/uk-strategy/index.htm). This Plan was produced at the request of UK Government rural affairs departments, in response to one of the major recommendations of the UK Country Report on Animal Genetic Resources, published in 2002. The Plan was produced by the National Steering Committee on Farm Animal Genetic Resources, which has members from conservation charities, industry, academia, government and other stakeholder groups.

The Plan considers:

- Why we need to protect our Farm Animal Genetic Resources (FAnGR), and how a National Action Plan can help.
- What, and where, are our Farm Animal Genetic Resources?
- How should we look after and use our FAnGR?
- What can Government do to help?

The Plan identifies 38 Recommended Actions to help in the protection and sustainable use of our FAnGR. These are broadly concerned with:

- Improving the collection, quality and availability of information and data on FAnGR to assist with their conservation and sustainable use;
- Supporting the prioritisation, development and implementation of projects to assist with the conservation and sustainable use of our FAnGR;
- Maintain a co-ordinating function and enhancing awareness of FAnGR issues in Government and industry.

The Recommended Actions, including the establishment of a UK Standing Committee on FAnGR, have been accepted by the UK Government rural affairs departments.

The Plan also identifies R&D needs, which include:

- Reviewing past molecular characterisation studies and identifying future priorities.
- Providing more objective breed characterisation, and developing methods for more effective targeting of characterisation studies.
- Producing robust guidelines on breeding nucleus flock/herd sizes to ensure the survival of a breed.
- Developing methods to quantify the degree of geographical concentration of breeds, and identifying breeds at risk as a result of geographical concentration.
- Investigating the costs and benefits of FAnGR to the rural economy.
- Investigating lifecycle nutrient efficiency of different breeds, and developing strategies to include this in within-breed selection.
- Developing a co-ordinated *in situ* and *ex situ* National FAnGR conservation strategy, including an evaluation of the costs and benefits of alternative approaches.
- Identifying potential new opportunities for FAnGR, e.g. by identifying breeds/breed characteristics of value for different types of conservation grazing.
- Identifying new opportunities for development of mainstream breeds in directions that support Government policy, such as reducing the environmental impact of livestock production, or helping to deliver animal health and welfare benefits.
- Developing user-friendly tools to assist in maintaining the 'genetic health' of mainstream or 'at risk' FAnGR.

The Plan is intended to develop over time, and BSAS members, along with all other stakeholders, are invited to help with its development and implementation. Comments are welcome via the Defra secretariat (c/o Phil Hambling, whose contact details are given above and in the Plan).

## Science of conservation for populations at risk

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Conservation is a long term activity, and the objectives of the activity must be clear and justified for it to be sustained in the long-term. Whilst the obligations for conservation-related activities are clearly set out in the Convention on Biological Diversity ("Rio" convention or CBD), the scale of activities will depend on the scope and quality of the case. A market-led justification for conservation of livestock is fraught with difficulty since if markets supported the full scope of existing genetic variation there would be no need for conservation. This does *not* imply that a long-term economic case cannot be made, but it does focus the arguments onto the future importance of the range of livestock breeds. Therefore beyond the benefits of visual diversity on the quality of life, there is a need for evaluating the scientific justification for conserving livestock breeds.

One approach leading from this rationale is to argue that since we do not know what is to be valued in the future then what must be done is to conserve maximum diversity, since this maximises the probability that the conserved sets contain a desirable extreme for the unknown quality. In livestock with its explicit structure of sub-populations in the form of breeds, the diversity observed can generally be divided into the amount of genetic variance observed between and within breeds. Whilst conserving both sources are an issue in livestock, the between-breed component has seen a dramatic reduction over recent decades primarily in breeds adapted to low and medium inputs. Whether this diversity should be assessed by DNA markers or by phenotypes has been an issue for debate particularly in the ecological literature (McKay and Latta, 2002), although it is perhaps more clear in livestock that much of the variation observed in readily available phenotypes will have been subject to selection and therefore potentially misleading for the purpose. The approach of core sets defined using DNA markers (Eding and Bennewitz, 2007) to give a guide to maximising diversity using different breeds, addresses at the same time the differential value of conserving breeds that may be comparatively inbred, so with only 1 variant per locus, or outbred, with potentially 2 variants per locus.

However the use of DNA is also problematical. A starting point for considering these issues (see Woolliams and Toro, 2007) is that the use of a small number of anonymous DNA markers to predicted relationship and inbreeding is open to substantial errors. This problem may have led to some serious shortcomings in the past, but these should be overcome by using the fruits of the genome sequencing projects in a number of livestock species, in particular the dense maps of single nucleotide polymorphisms (or SNPs) and the DNA chip technologies associated with them. From this point in time, studies of breed relationships based on heterozygosity and shared alleles across the genome will have $10^4$ or $10^5$ DNA data points for analysis. Such information also brings new opportunities. Past selection may leave footprints of selection formed by fixing of genes of large selective advantage, which might be either natural or artificial, with the footprint consisting of a chromosomal segment displaying reduced diversity within a sub-population. It remains to be seen how easy such footprints are to detect and how widespread they are in practice, but the question arises whether the conservation of diversity should prioritise using the pattern of diversity within the genome rather than a simple average over the entire genome.

It can be argued that we can be more pro-active in knowing what is of value to conserve for the future. For example one important set of attributes is resistance to pathogens in various forms. However there is a considerable gap in our knowledge of genetics of disease resistance between breeds or within breeds, with the possible exception of scrapie. One of the problems with unravelling this is the cost of obtaining good quality disease records from breeds kept together in the same environment and hence subject to the same disease challenges, either from industry or experimentally. Recent scientific developments may offer new ways to overcome such limitations, for example by the use transcriptomics in association with *in vitro* genetics.

In the above the focus has been on establishing the science for answering questions on which conservation activities may best meet our long-term objectives. However even when strategy has been mapped out there is still a need for science to underpin the implementation, and the design of the programme, so that it is adequate for meeting the long term objectives, and that implementation is not self-defeating by running the risk of introducing unnecessary bottlenecks for diversity. The science of implementation is considered by FAO (1998) and Roughsedge (this conference) will describe how it can be applied to the problems associated with establishing gene banks.

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# The role of gene banks in conserving farm animal genetic resources

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**Introduction** The concept of conserving genetic resources was first introduced in 1928 by Vavilov, a Russian botanist who founded a plant genetic resources bank at the All-Union Institute of Plant Breeding, Leningrad (Simon, 1984). The issue was first raised by the animal production world in 1959 where a need to conserve animal genetic resources was expressed at a Joint Symposium on Germplasm Resources for plant and animal breeders in Chicago (Simon, 1984). The emergence of these ideas can be associated with the recognition of advances in animal breeding and reproductive technology leading to a small number of highly productive breeds taking a dominant position in the production process. Three main forms of conservation can be considered; (i) cyopreservation (gene bank); (ii) maintaining control populations (no selection); (iii) managing live animal populations. This overview will focus on the role that cyopreservation plays in conservation of animal genetic resources.

**Planning** Undertaking *ex situ* conservation via a gene bank is neither a cheap nor easy undertaking. Therefore the first step in creating a new gene bank should be the clear definition of the purpose that it will serve. This process should ascertain whether the proposed gene bank will be fit for purpose. The purpose of gene banks falls into two broad categories; catastrophe aversion and support. Both roles can be viewed as risk management but the fitness for purpose requirements may differ. Further detail can be ascribed to the broad roles by addressing the question of what risk is being managed, such as the loss of specific genotypes in the case of the National Scrapie Plan Semen Archive (Roughsedge *et al*., 2006). More usually the role is to ensure that diversity is not lost, which in itself raises many questions. What species are we interested in? Are we interested in specific breeds? Are we trying to maximise within species genetic diversity? Do we take account of within breed genetic diversity in our quantification? Are we conserving a breed for cultural reasons? Having decided on the rationale it should become clear whether we are banking genetic material from mainstream breeds in addition to rare breeds. In the Netherlands two categories of collections have been clearly defined, the first category to avoid the risk of losing important genes or traits in commercial breeding programs, and the second to conserve rare breeds for their cultural and historic values. In France banked genetic material is classified as either representing breeds under threat, material from animals displaying extreme genetic characteristics or as a snapshot sample of any given breed. Currently the UK has collections of rare breeds (Rare Breeds Survival Trust) and geographically isolated breeds (Sheep Trust) in addition to the scrapie semen archive.

**Execution** In collecting genetic material to create a gene bank two questions must be addressed. The first is the way in which donor animals are going to be sampled and depends very much on the purpose of the bank. The second is the type of genetic material that is going to be sourced for the bank. Sampling must aim to minimise the relationship between donors regardless of any additional criteria such as the preservation of a specific genotype (e.g. Fernandez *et al*., 2006). The choice of conservation material depends on a variety of factors including economics, expertise, technology, reestablishment targets. The cost involved in collecting genetic material varies, with for example the collection of a DNA sample being relatively inexpensive and costs rising from semen to embryo collection. Using simulation Boettcher *et al*. (2005) showed that the combined banking of embryos in addition to semen greatly enhanced the chances of successfully re-establishing an extinct breed. Thus any economic appraisal of the cost of gene banking needs to consider the trade off between costs borne in the short term versus the cost involved in successfully utilising the collection in the future.

**Conclusion** In order that gene banks are able to deliver a genetic resource in a fit for purpose manner the feasibility of achieving the desired goals should be realistically assessed at the onset along with devising future release guidelines for stored material.

**Acknowledgement** Tim Roughsedge is grateful to SEERAD for financial support.

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# Niche market opportunities for rare livestock breeds

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**Introduction** Some of the many pig, sheep and cattle breeds in the British Isles are present in very small numbers and in only a few locations. The names of these rare/traditional breeds often reflect the place they originated from, e.g. Gloucesteshire Old Spots pigs. They are 'unimproved' in the sense that they have not been bred for greater production. The Rare Breeds Survival Trust (RBST) has an important role in the conservation of these breeds. The Traditional Breeds Meat Marketing Co Ltd (TBMM) developed out of RBST to market meat from them on the basis that one of the best ways to conserve breeds is to develop markets for their produce, making it financially feasible for farmers to maintain them. Consumers are often prepared to pay a higher price for the meat, which offsets higher production costs.

**Eating quality of traditional breeds** Underlying the increasing popularity of meat from rare and traditional breeds is a belief that the meat tastes better. The fact that it has been produced less intensively in a local food chain adds to the appeal. This paper considers the evidence for differences in meat quality between rare and modern breeds.

**Basis of variation in tenderness and flavour** Tenderness and flavour of the meat when eaten are probably the most important aspects of meat quality and reasons why variation occurs are shown in Table 1. Which of these could explain differences between modern and rare breeds? There is the possibility of a different genetic constitution ('tenderness genes'), perhaps associated with no selection for production traits. The generally higher fat level, good treatment of the cattle at slaughter and slow-craft style processing involving ageing on the bone, could also be important. For flavour, the older age of rare breeds, rearing on grass-based diets for ruminants and longer conditioning, probably 'on the bone', are likely to be important factors although the possibility of true genetic effects also exists.

**Comparison of eating quality in rare and modern breeds** **In Pigs** a study of Gloucestershire Old Spots (GOS), in comparison with Large Whites and pigs from two commercial breeding companies was made by Wood, Dransfield and Rhodes (1979). GOS had slightly thicker backfat compared with the other breeds, but a similar amount of fat in the carcass and similar eating quality. Warriss et al (1996) studied 11 pure breeds including 6 traditional British breeds. The traditional breeds had different muscle pH and colour characteristics compared with the modern breeds suggesting less rapid muscle metabolism at slaughter. Two of them (Berkshire and Tamworth) had high tenderness and flavour. A study by Wood et al (2004) compared pure bred Berkshire, Duroc, Large White and Tamworth breeds. The two traditional breeds, Berkshire and Tamworth, had the lightest, fattest carcasses but there were a few differences in the eating quality of the loin muscle.

**In Sheep**, Fisher et al (2000) showed that Soays finished on grass had a similar sensory profile to Suffolks finished on concentrates, with a lower lamb flavour, higher abnormal flavour and lower overall liking compared with grass-fed Suffolks. Soays had high scores for stale, rancid and ammonia which could be related to different fatty acids in muscle (more polyunsaturated) and more skeletal muscle substrates for proteolysis. In unpublished work, a similar flavour profile was observed in Portland sheep. This could denote genetic differences in meat flavour development.

**In Cattle,** a current project at Bristol involves a comparison between Longhorn and Charolais cross steers reared on unimproved grazing on a Somerset farm. Loin joints are aged for 21 days either "on the bone" or in vacuum packs. The results show that Longhorns had higher scores for tenderness and beef flavour than Charolais and that ageing on the bone produces higher tenderness and flavour than "wet ageing" in vacuum packs.

**Table 1** Factors affecting meat tenderness and flavour

| **Tenderness** | **Flavour** |
|---|---|
| Decreases with age. | Increases with age in beef and lamb (the greater flavour intensity of older lamb/mutton not to all tastes). |
| Lower in bulls than steers. | |
| Improved by steady growth. | |
| Improved by fat level, especially in muscle Tenderness genes. | Major diet effects e.g. grass-fed beef and lamb. |
| Greatly affected by post-farm gate factors e.g. considerate handling and stunning, longer hanging/conditioning time, slow chilling. | Processing has a role e.g. conditioning time/temperature. |

**Conclusion** Meat from traditional livestock breeds reared and processed in traditional ways is attractive to increasing numbers of consumers for many reasons. There have been few detailed studies of eating quality in traditional breeds although it is often firmly stated that the meat will be more tasty. Evidence collected at Bristol shows that tenderness and flavour are at least as good and often better in traditional compared with modern breeds of sheep, cattle and pigs. In sheep, there is evidence for unusual flavours in some breeds, probably emanating from the fat and muscle tissue.

# Conservation grazing

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'Conservation grazing' is normally interpreted to mean livestock grazing for wildlife conservation purposes. Farm livestock grazing is essential for the management of many of the United Kingdom's most important habitats. For example, permanent grassland, heathland, wood pasture, floodplain and coastal marshes all require some grazing to maintain the structure and composition upon which a wide variety of wild plants and animals depend for their survival. Farming activities have played a significant role in shaping these habitats over time and the continuation of certain livestock husbandry practices is often crucial for their sustained existence. The key livestock species utilised for conservation grazing are cattle, equines (mainly ponies), sheep, goats and occasionally pigs. Wild herbivores (e.g. deer and rabbits) may also play a significant part in conservation grazing outcomes.

Livestock grazing (and/or browsing) for conservation objectives can maintain species-rich habitats by controlling more aggressive species which would otherwise dominate these areas and by preventing scrub encroachment. Such grazing also removes plant material more gradually than mechanical cutting or burning and provides mobile fauna with a better opportunity to migrate to other suitable ecological niches. In addition, conservation grazing can encourage haymaking which promotes active management of valuable meadow habitats.

However, conservation grazing itself needs to be as sustainable as possible. Its sustainability should be achieved environmentally, economically, socially, culturally and even philosophically. Environmentally, the two principal problems can be encapsulated generically as overgrazing and undergrazing (or complete withdrawal of grazing). To make conservation grazing robust in these terms it must be engaged in delivering a broader range of public benefits than simply providing a special habitat management service. This type of grazing can also make valuable contributions to the conservation and enhancement of natural resources (e.g. soils and water), genetic resources (e.g. farm animals and cultivated plants), landscapes, local economies, local communities and our historic heritage.

Successful conservation grazing requires six 'rights':

1. The right type(s) of livestock: For instance, consider the appropriateness of livestock species (single or mixed), breeds and crosses, breeding history, ages and weights, hefting/acclimatisation and local adaptation, feeding and nutrition, hardiness and temperament.

2. The right numbers of such livestock: Grazing at stocking rates to achieve sward and habitat condition results; avoidance of poaching, overgrazing, undergrazing, and other damage to the land or water bodies; minimisation of requirements for inorganic and organic fertilisers, chemicals, medicines, bulk fodder and other supplementary feed.

3. Grazing at the right time(s): Sensitive to weather, ground, public access conditions; restrictions to specified period(s) of each year; rotational grazing (annually, within year or every few years).

4. Grazing in the right places: Where it is beneficial to land, vegetation, habitats and species; with minimum transportation of livestock; where no insurmountable conflict with public access; where hazards properly assessed.

5. With the right livestock husbandry: High standards of animal health and welfare must be maintained; livestock keepers must have adequate skills/experience/training.

6. With reasonable financial returns: From primary livestock products (e.g. beef, milk, lamb, wool, etc.); from sales of breeding livestock; from high level environmental land management schemes (e.g. Higher Level Environmental Stewardship scheme – Native Breeds at Risk grazing supplement); from associated diversification enterprises.

The future of conservation grazing will depend upon national (UK) and international (EU) agricultural and other rural policies, global markets for livestock and their products, local processing facilities, local marketing opportunities for distinctive products, the availability of suitable farm [grazing] animal genetic resources, economic returns to grazing livestock keepers and acknowledgement of the diverse public benefits resulting from grazing systems that deliver multi-faceted conservation.

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## "Agriculture at a New Dawn: Food Security v Energy Security"

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The Post War era has seen UK agriculture experience mixed fortunes. Following the scarred memories of food shortages farming experienced one of its regular cyclical booms in the sixties and seventies with guaranteed prices that pushed forward production to improve the level of self sufficiency demanded by the politicians. In continental Europe this encouragement was provided by the emerging Common Agricultural Policy (CAP) which Britain joined in 1973. The CAP promoted greater food production by different methods based on intervention buying at guaranteed prices when there were surpluses only to release the products back on the market at times of shortage. This system worked effectively while the periods of excess were smaller than the periods of surplus. The eighties saw the reversal of this pattern and the establishment of unacceptably high levels of intervention stocks of all major commodities. The system became unworkable and although the French and Germans fought to retain the status quo, the old CAP had become not only indefensible but critically not in the best interests of either consumer or farmer.

Progressive change has been introduced with a succession of reforms culminating in the 2003 Fischler reforms which effectively introduced decoupling of support from the unit of production. This liberalisation has the potential to have major effects in the market place as farmers turn their almost absolute focus from maximising their returns from the support mechanisms to focusing much more clearly on the market and what opportunities exist to improve their returns. This substantial change will be consolidated by further reforms in 2008 which are likely to see the abolition of set aside and milk quotas.

While this change is significant and critical to the future prosperity of farming in general and in the UK specifically, it is far from the only driver for change that we are currently facing. Indeed the level of change that is on our threshold offers up enormous opportunities to UK farmers, in particular, in the coming years, but only if the industry is prepared to recognise the potential of the change and take on the challenges that come with it.

Climate Change is in the news almost every day, but what is often not reported is that the effects of climate change are not consistent around the world. There will be winners and losers and on the assumption that we retain the Gulf Stream, then the British Isles and parts of central Europe through from Northern France, Southern Germany to western Hungary will come off relatively well with similar rainfall levels albeit in a changed pattern. And it is rain, or rather in most cases the lack of it, that is to become a major factor for society as a whole and one that will limit our ability to produce crops from the land mass available. Water shortages in the Iberian peninsula are already evident as they are in parts of the USA, China, India and throughout Australia.

This constraint on our global production potential will be compounded by two other major factors. First an increasing and increasingly wealthy population in China, India and the Asian continent which will have the resource to buy food and demand a higher standard of living based around meat rather than a vegetarian diet. These changes are already evident as these countries feed the labour demands of their industrial revolutions by moving large populations from rural subsistence farming to urban dwellers, with greater disposable incomes, which need to be fed.

And secondly we will have the demands on the land as a potential source of renewable energy as we go through the period of peak oil production and experience increasing fears in Western Europe on the dependence of our gas supplies from ever farer east and through countries with political masters who at best can be described as unreliable. This is without even considering the need to diversify to renewable energy as a means of mitigating our increasingly seriously damaging effects on the atmosphere.

So all this adds up to the potential for enormous change to deliver a new dawn for UK agriculture with the potential to expand and prosper once again. It will need to be able to exploit opportunities and develop new technologies to maximise economic output while maintaining a clear guardianship of the environment in a long term sustainable manner. Agriculture will become a much more diversified business with the need to create output not only for food needs, but also for energy and for the environmental and recreational needs of our society as a whole.

This is the challenge that we will have to take on and turn to our advantage. It will not be for the faint hearted, but it will offer a new and invigorated dynamism that delivers real sustainability in every meaning of the word.

# Why Agriculture in the UK is as it is

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Agricultural activity is determined by the simultaneous operation of four underlying exogenous forces, natural resources, markets, science and social values. All these forces have been changing and the UK agricultural industry, including not only farms but all the businesses involved in turning raw materials into food and other consumer products are compelled to adapt. An account of agriculture today is in practice a single frame of a moving picture.

This paper describes how these forces have shaped the industry. It provides a snapshot, mainly in terms of economic dimensions, about the current size and structure of the industry, its output and its role within the wider economy. It then discusses how continuing changes in the underlying variables are reshaping of the industry. This provides a background for later papers that discuss how the industry may respond and how this will affect those who buy its products.

# Enhancing crop-livestock systems for agricultural productivity, food security and reduced poverty in developing countries

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Mixed farming systems, in which crops and livestock are integrated on the same farm, are the backbone of small-scale agriculture in most developing countries (Lenné and Thomas, 2006). Crops and livestock contribute in a diversity of ways to enhancing the livelihoods of the poor through provision of food, income, draught power and employment. Livestock are a major source of high-quality protein, minerals, vitamins and micro-nutrients for developing country populations and livestock-derived food items contribute significantly to agricultural GDP. Animals also play a major role in improving food security in such countries, because cash income obtained from the sale of animals is regularly used to buy non-livestock food items and inputs to farming. It is predicted that the demand for livestock products in developing countries will increase substantially over the next 25 years (Delgado et al., 1999). Failure to meet the challenge of further growth in the livestock sector in these regions is likely to result in the growing urban demand for livestock products being met by subsidized imports. This will be to the detriment of small-scale producers and national and regional economic growth.

Developed country governments have strongly oriented their international development policy towards achieving the Millennium Development Goals (MDGs) by 2015. Research on crop-livestock systems in developing countries can contribute to the MDGs by:

- increasing income generation for poor small-scale farmers from sale of livestock products;
- improving nutrition for the poor, especially infants and children, through increased availability of milk and other livestock products; and
- through sound intensification strategies for crop and livestock production that will allow the same area of land to be more productive while sustaining the environment

In recent years, many donors and the international research community have made major investments in increasing crop productivity, improving fodder production and increasing livestock productivity in priority production systems in developing countries to the benefit of the poor. To date, however, full realization of the benefits from this substantial research effort has been hampered by the historical lack of cross-disciplinary linkages and cross-sectoral approaches: research on crops and livestock has been removed from its integrated systems context. Research effort is spread amongst a plethora of institutes and projects and there is great diversity in the extent to which different end-users and delivery agents are integrated, to ensure that research is both relevant and deliverable to farmers. Clearly, this investment in past research needs to be better integrated and linked more directly to current and future challenges if it is to further contribute to food security, reducing poverty and enhancing livelihood opportunities for the poor.

The main aim of this paper is to show how knowledge and technologies generated from past research are directly relevant and immediately applicable to enhancing the contribution of crop-livestock systems to agricultural productivity, food security and reduced poverty in developing countries. The paper will give emphasis to advances made in the development and promotion of dual-purpose food-feed crops, improving the quality of on-farm feed resources through managing crop diseases, pests and weeds, improved and enlightened use of available systems-based feed resources for ruminants and non-ruminants, development and promotion of fodder conservation strategies, development of seed systems for food-feed crops, improving marketing systems for livestock products and fodder and policy issues (Lenné and Thomas, 2006). It will also highlight the importance of knowledge and technology spill-overs from one region to another. Spill-overs have been a pervasive feature of agricultural development and in recent years have been responsible for up to half of the agricultural productivity gains (Alston, 2002).

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# Robustness in dairy cattle

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**Introduction** Robustness can be defined as "the capacity to handle disturbances in common and sustainable, e.g. economically, systems". To achieve a robust farming system, a broad perspective is needed (Napel 2005), but here we focus on genetic selection for robust cows and the origin of the need for such animals.

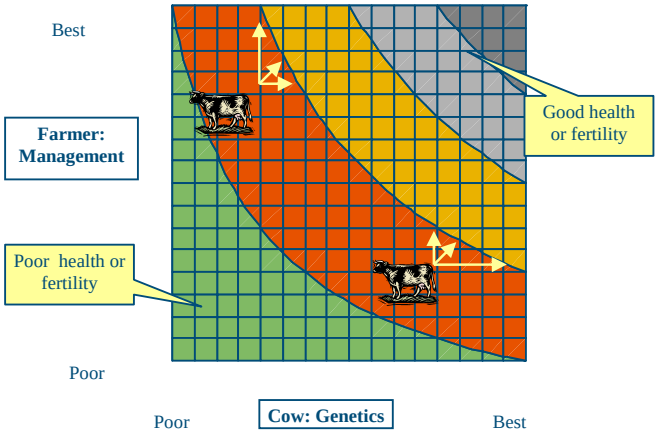


**Figure 1** Schematic demonstration of the interaction between management and genetics in relation to health and fertility

**Robust cows** The interest in more robust cows started in the mid 1990s when awareness grew that selection for milk yield alone was associated with poorer health and fertility. To date we assume that these effects are a consequence of pleiotropic effects, rather than effects of yield per se (Beerda *et al.*, 2007). For some years, breeding indices typically account for health, fertility and longevity traits. Still dairy farmers call for more robust cows as exemplified by the interest in cross-breeding and other breeds. Possibly, because even though index weights for robustness traits are high, accuracy of selection is often low compared with the accuracy of selection for milk yield. Also, the perceived lack of robustness, like poor fertility, may reflect specific problems, for example resource allocation associated with selection for yield (Veerkamp *et al.*, 2003). Here, we put forward that the call for robust cows is most likely a consequences of pressures on labour and prices on one hand, and increasing demands for health and welfare on the other hand (see Figure 1). If management is restricted, e.g. as actions are constrained by prices, labour availability or regulations, the most effective way to improve health or fertility appears to be through the cows' genetics.

**Genetics of robustness** If genotype by environment interaction (GxE) is a major determinant of robustness then also the relation between genetic variances and the environment should be taken into account. Calus (2006) estimated GxE for several health and fertility traits, using many different environmental descriptors. The G×E effects consisted mainly of heterogeneous genetic variances with limited reranking. Of the many environmental parameters those linked to nutrition and energy balance caused the strongest G×E. The implication of heterogeneous variance was that selection for fertility will yield higher selection responses in herds with on average poorer fertility, and that selection for reduced SCS will yield higher selection responses in herds with on average higher SCS. Hence, for those herds that are constrained to improve their management genetic selection is most effective, which contrasts to the popular believe of "getting your management sorted out first".

**Selecting robust cows** Establishing more effective selection for health and fertility traits is the obvious way to improve robustness. A second way can be to take account of GxE in selection and a third to select for robustness directly. For example, by selecting against environmental sensitivity (ES) for health and fertility, with ES as the slope of an animals reaction norm (Falconer 1990). It is still debatable whether or not low ES is a desirable trait. Because when the environment becomes 'more supportive' it is expected also that management changes are translated into practice. Some studies indicate that in a continuously improving environment, selection for increased performance leads to increased ES for the trait selected on (i.e., reduced robustness). We found that selection for yield is expected to increase ES for yield, and selection for fertility is expected to decrease ES for fertility (Calus 2006).

**Conclusion** Dairy farmers demand for more robust cattle, and the first steps in this direction have been made by incorporating health and fertility traits in breeding indices. The accounting for GxE interactions and direct selection for robustness as a trait needs more consideration .

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# Robust Chickens: Improving productivity, health and welfare by including social effects in the selection decisions

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**Introduction** The usual approach in livestock genetic improvement is to consider the phenotype as a sum of a heritable component and a residual non-genetic component, referred to as environment, P = A + E. However, when individuals are kept in groups, the environment that an individual experiences contains a social component due to its group members. In contrast to the physical environment, this social component may have a heritable basis and therefore respond to selection. Thus, for traits affected by social interactions among individuals, current selection strategies can be improved by including the social effects of individuals in the selection decisions. Here we present some basic theory for improving traits affected by interactions, and first empirical results in three populations of layer chickens.

**Methods** When traits are affected by interactions among individuals, the usual model, $P = A + E$, needs to be extended to include social effects, giving $P_i = A_{D,i} + E_{D,i} + \sum_{i \neq j}^{n} A_{S,j} + \sum_{i \neq j}^{n} E_{S,j}$, in which $n$ is group size, $A_{D,i}$ and $E_{D,i}$ are the usual $A$ and $E$, $A_{S,j}$ denotes the heritable social effects of group members $j$ on the phenotype of individual $i$, and $E_{S,j}$ the non-heritable social effects. In this situation, total improvement of the trait value equals $\Delta P = \Delta A_D + (n-1)\Delta A_S$, and the total additive genetic variance in the traits equals $\sigma^2_{A_D} + 2(n-1)\sigma_{A_{DS}} + (n-1)^2\sigma^2_{A_S}$, in which $\sigma^2_{A_D}$ is the usual additive genetic variance (Bijma *et al*., 2007a). This result shows that presence of social interactions among individuals may substantially increase the total heritable variance, in particular with large groups. Consequently, prospects for improvement of traits affected by interactions, such as mortality due to cannibalism in laying hens, may be substantially better than currently perceived. The additive genetic variance involved in this model can be estimated by generalizing animal models for maternal effects to any type of interaction among individuals (Bijma *et al*., 2007b).

**Results** To investigate the importance of heritable social effects, the above model was fitted to data on mortality of three commercial lines of chicken provided by Hendrix Genetics. Lines consisted of 3,000 to 6,000 non-beak trimmed individuals kept in 4-bird wire cages, and average mortality per line varied between 30 and 50%. Conventional data analyses with an animal model revealed heritabilities for survival in the usual range of 3 to 9%. Analyses accounting for social interactions, however, yielded heritabilities that were approximately three-fold greater for all three lines. One line showed a clear negative genetic correlation between direct and social effects, indicating that classical selection for survival would increase competition between individuals (i.e. “aggressiveness”). In both other lines the genetic correlation did not differ significantly from zero.
Investigation of the consequences for response to selection for one of the three lines gave the following results. The classical prediction equalled an improvement of 7.8 days of survival. Response predicted for the optimal selection method equalled 23 days of improvement, a value approximately three-fold greater than the classical prediction. The optimum selection method had groups consisting of full sibs and a selection criterion that consisted of approximately 50% emphasis on individual performance and 50% on group performance. Those results indicate that accounting for social effects in genetic analyses and selection decisions is highly relevant when trying to improve survival in non-beak trimmed laying hens, and that prospects for improvement are good.
To further substantiate the relevance of social interactions for genetic improvement, a selection experiment was undertaken that specifically utilized the heritable variation in social effects. Response to a single generation of selection was substantial, in line with the genetic parameters estimated from the full analyses, and clearly larger than what could be explained from the conventional heritability. There were no trade-offs for production traits.

**Conclusion** Social interactions among individuals give rise to extra heritable variation in traits, which is hidden from classical analyses. The importance of social interactions for specific cases can be determined by using a simple extension of current animal models, which can be implemented in standard VCE software. Presence of heritable social interactions requires changing our current selection criteria, but this can be implemented in a straight forward manner (not shown). The results for the chicken population illustrate the increased potential for genetic improvement when heritable social interactions are present.

**Acknowledgements** Hendrix Genetics is acknowledged for providing data.

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# Regulation of gene transcription by fatty acids

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Dietary lipids have the capacity to regulate many aspects of metabolism in a manner depend on chain length and the number, and position, of double bonds in the fatty acids they contain. For example, while long chain saturated fatty acids increase plasma cholesterol, polyunsaturated fatty acids (PUFA) have the opposite effect. PUFA also have a major impact on the activity of enzymes associated with carbohydrate metabolism and lipid biosynthesis and oxidation. The role of fatty acids as substrates in these pathways, and their conversion to eicosanoids, with different levels of activity depending on the parent molecule, represents two mechanisms whereby they exert such effects. However, more recently it has been established that fatty acids and/or their derivatives also have direct effects on the expression of genes for proteins regulating carbohydrate and lipid metabolism.

For a nutrient to regulate gene expression there is an assumption that it, or a derivative, must accumulate within the cell in proportion to the amount consumed. While it is clear that the bulk of fatty acids are either esterified or oxidised, there is growing evidence that fatty acid binding proteins may indeed transport fatty acids or their coenzyme A derivatives into specific compartments of the cell, including the nucleus. Changes in the size and composition of these pools of fatty acids may regulate gene expression by modulating transcriptional and posttranscriptional events. In particular, much recent research has focused on the impact of fatty acids and their derivatives on the activity or abundance of specific transcription factors involved in regulating the expression of genes for key enzymes, receptors and transport proteins. Such transcription factors include, the sterol regulatory element binding proteins (SREBPs), the liver X receptor (LXR), the peroxisomal proliferator- activated receptors (PPARs) and hepatic nuclear factor 4α (HNF4α). It is also possible that fatty acids regulate gene transcription through modulating the activity of various co-activators and co-repressors that control the activity of these transcription factors.

The SREBPs play a major role in regulating the expression of genes for proteins associated with lipid and lipoprotein metabolism. These proteins are synthesized in an immature form on the endoplasmic reticulum and are activated by proteolytic cleavage and release of a truncated mature form, which migrates to the nucleus and regulates expression by associating with a specific nucleotide sequence (the Sterol Regulatory Element, SRE) on the promoter of target genes. Two SREBP genes produce three separate proteins (SREBP1a and 1c from one and SREBP2 from the other). SREBP1c predominantly regulates the expression of genes coding for lipogenic enzymes, SREBP2 those associated with cholesterol metabolism and SREBP1a has the capacity to regulate both fatty acid and cholesterol metabolism. Accumulation of cholesterol in the cell inhibits the proteolytic release of SREBPs from the endoplasmic reticulum and thereby down–regulates the expression of target genes. However, PUFA have also been shown to reduce the expression of SREBP1c-regulated genes in the liver. While the exact mechanism(s) remain to be fully elucidated, this may involve another transcription factor, namely LXR, a ligand –activated nuclear receptor that regulates a variety of genes involved in lipid metabolism including SREBP1c itself. It has been suggested that PUFA interfere with the interaction of LXR with its ligand (probably an oxygenated derivative of cholesterol) and thereby reduces activation of the SREBP1 gene.
The PPARs are a class of nuclear receptors that regulate expression of a wide range of proteins involved in lipid metabolism. Three isoforms, PPARα, β(also known as δ) and γ are expressed in a tissue specific manner. PPARα is the major isoform in liver while PPARγ predominates in adipose tissue and PPARβ is expressed more ubiquitously. Each of the PPARs are activated by a wide range of ligands including some fatty acids. Of these the most potent are the long chain omega-3 PUFA such as docosahexaenoic acid (DHA). In rodents, pharmacological activation of PPARα induces peroxisome proliferation and results in dramatic increase in fatty acid oxidation. While many species, including human, do not exhibit such peroxisome proliferation, it is still believed that, at least part of, the hyotriglyceridaemic effect of long-chain omega-3 PUFA is due to their role as ligands for PPARα and consequent up -regulation of genes for enzymes involved in fatty acid oxidation.

While SREBPs and PPARs have been shown to be regulated by PUFA, identifying specific transcription factors that are regulated by saturated fatty acids has been more challenging. There is some suggestion that in the liver HNF4α may be activated by coenzyme A derivatives of fatty acids. This appears to be in dependent on the chain length and degree of saturation, with palmitic acid (C16:0) being the most potent. HNF4α regulates expression of many hepatic genes including various apolipoproteins and the microsomal triglyceride transfer protein (MTP) which is essential for the synthesis and secretion of very low density lipoprotein. More recently saturated fatty acids have also been shown to regulate gene expression through altering the expression of the co-activator protein, PGC-1β. This protein interacts with SREBP1c when it is associated with the promoter of its target genes and enhances the activity of the transcription factor. How saturated fatty acids increase PGC-1β and the relative importance of this in mediating the effect of saturated fatty acid requires further study.

Much of the evidence described above is derived from cell culture studies, where single fatty acid entities can be added and effects measured. Translating these to an *in vivo* situation when complex mixtures of fatty acids are normally consumed and join the complex pool of fatty acids and derivatives already present in the body, represents a major challenge for future research.

# Growth and nutrition of cattle early in life: long-term consequences for beef production

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**Introduction** This paper reviews research on consequences of cattle nutrition and growth during foetal and neonatal life for subsequent growth, efficiency, carcass, yield and beef quality characteristics (Greenwood and Cafe 2007). It includes findings from our recent studies on consequences of growth during pregnancy and to weaning (Greenwood *et al.* 2006). The reader is also referred to reviews on consequences of prenatal development in livestock by Bell (2006), and on consequences of *bovine* foetal, pre-weaning and early post-weaning nutrition and growth by Greenwood *et al.* (2005).

**Growth and efficiency** Severe, chronic growth retardation of cattle early in life is associated with reduced growth potential, resulting in smaller animals at any given age. The capacity for long-term compensatory growth diminishes as the age of onset of severe nutritional restriction resulting in prolonged growth retardation declines, such that more extreme intrauterine growth retardation can result in slower growth throughout postnatal life. Neither restricted growth *in utero* or from birth to weaning influenced efficiency of nutrient utilisation later in life.

**Carcass characteristics and yield** Retail yield from cattle severely restricted in growth during pregnancy or from birth to weaning is reduced compared to cattle well-grown early in life, when compared at the same age later in life. However, retail yield and carcass composition of low and high birth weight calves are similar at the same carcass weight. At equivalent carcass weights, calves that are grown slowly from birth to weaning have carcasses of similar or leaner composition than those grown rapidly. However, if high energy, concentrate feed is provided following severe growth restriction from birth to weaning, then at equivalent weights post-weaning the slowly-grown, small weaners may be fatter than their well-grown contemporaries.

**Beef quality and myofibre characteristics** Restricted prenatal and pre-weaning nutrition and growth do not adversely affect measures of beef quality including shear force, compression, cooking loss and colour. Similarly, *bovine* myofibre characteristics are little affected in the long-term by growth *in utero* or from birth to weaning, despite specific myofibre type-related effects at birth and weaning, respectively.

**Interactions between prenatal and pre-weaning growth** Interactions were not evident between prenatal and pre-weaning growth for subsequent growth, efficiency, carcass, yield and beef quality characteristics, within our pasture-based production systems.

**Interactions with genotype** A major objective of our research has been to determine the extent to which genotype may interact with nutrition early in life to influence productive characteristics. To achieve this objective, our research included offspring of Piedmontese (a high muscling and higher birth weight breed, homozygous for a mutation that produces non-functional myostatin) and Wagyu (a high marbling or intramuscular fat and lower birth weight breed) bulls mated to Hereford cows. Perhaps surprisingly, no interactions between sire-genotype and growth early in life were evident for any growth, efficiency, carcass, yield and beef quality parameters.

**Conclusions and speculation** We propose that within pasture-based production systems for beef cattle, the plasticity of the carcass tissues, particularly of muscle, allows animals that are growth-retarded early in life to attain normal composition at equivalent weights in the long-term, albeit at older ages. However, the quality of nutrition during recovery from growth retardation during early-life may be important in determining the subsequent composition of young, light weight cattle relative to their heavier counterparts.

**Potential future research** Finally, it needs to be emphasised that long-term consequences of more acute environmental influences during specific stages of foetal and neonatal calf development remain to be determined. This need extends to consequences of nutrition and growth early in life for reproductive capacity.

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## Responses to Nutrients in farm animals: implications for product quality

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In the context of increased globalisation and competitiveness, producers of animal products have been the most affected with considerable reductions in profit margins. Research on nutrition in farm animals is thus still needed to reduce the costs of production by increasing metabolic efficiency. To achieve this goal, the objective is always to control animal performance accurately by improved quantification of animal requirements and by precise feed evaluation. At the same time, the farming and agri-food sectors are faced with a general saturation of food markets in Europe and with an increasing demand by consumers for high-quality meat and dairy products. This has also led to specific research in nutrition which aims to optimise metabolic activity of muscle and mammary gland to produce meat and dairy products of the desirable composition. This paper aims to address this important question: how animal nutrition may help to optimise metabolic efficiency and product quality. Today this needs better knowledge of tissue and organ requirements and of nutrient fate within tissues and organs as well as of their contribution to the quality of animal products. Furthermore, in order to achieve this goal of greater understanding of animal response to nutrition, new concepts and techniques are available to decipher mechanisms that were impossible to address adequately a few years ago. In this connection, emerging approaches such as genomics and modelling provide the means for a better insight into the mechanisms which regulate metabolism at tissue or whole body level.

The partitioning of nutrients among tissues and metabolic pathways serves to maintain a steady supply of energy and amino acids to meet the tissue needs. Depending on nutritional level and diet composition, the splanchnic tissues have a profound quantitative and qualitative impact on the supply of nutrients to muscle and mammary gland. Therefore, in the future, nutritionists should be able to formulate diets to meet the specific nutritional requirements, on the one hand, of the gut and the liver and, on the other hand, of targets tissues and organs (muscle, udder) taking the transformation and recycling of nutrients by the splanchnic bed into account.

Whichever the tissue or the organ, metabolic enzymes are regulated by nutrients through short-term regulation of their activities and long-term regulation of expression of their genes. Liver is characterized by high anabolic and catabolic rates in relation with high enzyme activities. Consequences of liver metabolic regulation on energy and amino acid supplies to muscles may affect protein deposition (and hence growth) as well as intramuscular energy and nitrogen stores. Adipose tissues are important body reserves of triglycerides, which result from the balance between lipogenesis and lipolysis. Consequences of liver and adipose tissue metabolic regulation on fat turnover may affect fatty acid metabolism in muscles and in the mammary gland, and hence the quality of meat and milk. These processes depend on the feeding level and on the nature of the diet, which have an indirect quantitative and qualitative effect on nutrient delivery to muscles and mammary gland.

Skeletal muscle is of major economic importance since it is finally converted to meat for consumers. An increase in meat production with low costs of production may be achieved by optimizing muscle growth, whereas high meat quality requires, among other factors, the optimization of intramuscular glycogen and fat stores. In muscles, the regulation of rate-limiting nutrient transporters, metabolic enzyme activities, ATP production, and protein turnover (mainly through regulation by amino acid fluxes and insulin action) as well as the interactions between nutrients, affect free energy availability and protein deposition for muscle growth. These processes also modify muscle metabolic characteristics which determine meat quality. Similarly, the mammary gland produces milk which is consumed by humans directly or after transformation. This production is determined by the number and the activity of mammary epithelial cells, the function of which is subject to complex hormonal regulation. Furthermore, milk composition, which determines the nutritional properties of milk products, is affected in particular by nutrition. Thus, milk lipid composition is modulated by both the level and the composition of the animal diet, through *de novo* synthesis and circulating lipid uptake by the mammary gland.

So both muscle and udder metabolisms are regulated by feeding level and diet composition, and also by the general metabolic status of the individual, which is hormonally regulated. Diet composition determines the nature of available nutrients and the rate of nutrient delivery to tissues and organs, thereby regulating metabolism. This aspect has been illustrated firstly by studying the effects of the changes in dietary nutrient supply which occur at weaning. These changes differ between monogastric mammals and ruminants, leading to specific modes of metabolic regulation. In the same way, regarding the differences in response to nutrients between species, bovine and caprine differ with respect to the impact of forage/concentrate ratio with or without fat supplementation on their milk fat. In addition, the regulation of novel genes depending on the diet has been demonstrated. The effect of feeding level has been illustrated by studying the effect of diet restriction followed by a period of compensatory growth in young animals. Muscle-specific adaptations were demonstrated. Consequently, rearing systems may be extremely important during postnatal life.

In conclusion, research is now directed towards the determination of tissue and organ nutrient requirements, and a better knowledge of nutrient partitioning between and within organs and tissues. The discovery of key molecules which regulate metabolism (e.g. leptin, adiponectins) and of new molecular mechanisms by exploiting more powerful techniques (genomics, metabolomics) will help to achieve these objectives. Integration of the different levels of knowledge with the help of modelling tools will finally allow scientists to formulate new types of diets capable of achieving production of high quality products with lower costs of production.

# Minimising diffuse pollution from livestock manures – the challenges ahead

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In the region of 90 million tonnes of farm manures, supplying 450,000 tonnes of nitrogen (N) and 119,000 tonnes of phosphorus (P) are applied to agricultural land in the UK each year. These applications are a valuable source of plant nutrients but they are also recognised as the largest source of controllable pollution in present day farming systems. Over 70% of nitrate entering water systems is estimated to originate from agricultural land and the transfer of particulate and soluble P from agricultural land has been estimated to contribute 40-50% of P loads in watercourses. Ammonia ($NH_3$) emissions from UK agriculture contribute *c.*80% of total UK emissions with losses following the land spreading of farm manures responsible for *c.*96,000 tonnes of $NH_3$ each year

The UK is committed to reducing diffuse pollution under a number of EC Directives and International protocols. The Water Framework Directive (2000/60/EC) requires Member States to protect, enhance and restore all bodies of surface water (including rivers, lakes, estuaries and coastal waters) and groundwater with the aim of achieving good ecological status by 2015, subject to certain exemptions set out in the Directive. The Nitrate Vulnerable Zone (NVZ) Action Programme (introduced in 1998 and amended in 2002 as part of the UK's commitment to the EC Nitrates Directive) currently covers 55% of agricultural land in England, 3% in Wales and 14% in Scotland. The NVZ Action Programme prevents the application of high available N manures (i.e. pig and cattle slurry and poultry manures) in the autumn on light and shallow soils, and limits manure N loadings to land. As part of the National Emissions Ceilings Directive, the UK is committed to reduce annual ammonia emissions from 320,000 tonnes $NH_3$ to 297,000 tonnes $NH_3$ by 2010. The improved management of manures at land spreading has a key role to play in reducing emissions with bandspreading and shallow injection slurry application typically reducing ammonia emissions by *c.*30-70% compared to conventional surface broadcast applications.

In order to safely manage manures and the nutrients they contain in an environmentally sustainable way, it is important to apply manures at agronomically required rates (i.e. within an integrated fertiliser plan) to growing crops and at times that minimise drainflow / leaching and surface runoff risks. This simple set of rules, however, requires appropriate knowledge and farm infrastructure. For example, to apply manures at agronomically required rates, it is necessary to know the soil nutrient status, the crop demand for nutrients, the manure nutrient content and availability, and to spread the manure at a known rate uniformly across the field. On many farms, changing manure application practices to reduce nitrate leaching and phosphorus losses will require significant financial investment in extra slurry storage capacity to avoid the need to apply slurry in the autumn / winter. In addition, investment in slurry bandspreading equipment is likely to be required to apply slurry evenly to growing arable and grassland crops in spring/summer, without causing damage to soils and reducing crop quality.

Recent studies on free draining soils, have shown that late spring/early summer cattle slurry applications before second cut silage can increase ammonia emissions by up to 3-fold compared with autumn to early spring timings. Similarly, on arable land, moving slurry applications from autumn to spring on winter cereal crops reduced nitrate leaching losses, but increased ammonia emissions by up to 4-fold. Other studies on drained clay soils have shown that changing slurry application timings from autumn to spring can reduce nitrate leaching losses, but lead to increased losses of phosphorus and ammonium-N in drainage waters because of rapid movement of slurry nutrients through soil macropores, via so called 'by-pass' flow.

This paper will outline the need for an integrated approach to livestock manure management to ensure that measures to reduce the loss of one pollutant do not exacerbate losses of another (so called 'pollution swapping'). The need for capital investment in farm infrastructures to reduce diffuse pollution will also be discussed.

# Livestock and water in developing countries with an emphasis on Sub-Saharan Africa

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**Introduction** Projected increased demand for food in developing countries over the next 30 years implies a correspondingly great need for additional agricultural water unless integrated research and development can achieve much higher water-use efficiencies. Without appropriate innovations in water management, poor access, quality and supply will continue to constrain food production. A global consortium recently completed the *Comprehensive Assessment of Water Management and Agriculture* (CA 2007) and identified many options for overcoming water-related constraints to sustainable food production in developing countries. Historically, research and development of water resources has neglected the potential benefits and impacts of livestock. Apart from drinking water, livestock professionals have not given adequate attention to the use of and impact of domestic animals on water and related environmental health. In the absence of good science, popular literature is often highly critical of livestock production because of its perceived excess depletion of vital water resources. The CA uniquely attempted to address this issue (Peden 2007). This paper summarizes the CA's findings about livestock for the benefit of the British Society of Animal Science (BSAS) and the wider livestock research community.

**Livestock water productivity (LWP)** is the ratio of the net beneficial animal products and services produced in an agricultural production system to the amount of water depleted as a cost of producing them. Production system scales can vary in size ranging from farms and fields to watersheds and river basins. Depleted water is water lost from production systems such as evaporation, transpiration and downstream discharge. Figure 1 presents a simplified version of the LWP assessment framework (Peden 2007) used to estimate the amount of water depleted in diverse livestock systems. While much is known about drinking requirements of animals, direct consumption of water does not contribute to water depletion because water drunk remains in the production system even though drinking may be vital to animal survival. Strategic feed sourcing, conserving of water and enhancing animal productivity provide multiple options for increasing LWP. The first two strategies help ensure that feed and pasture supplied to animals makes best use of available water and, where appropriate, shifts water depletion pathways from unwanted run-off or discharge and evaporation to transpiration and infiltration. The productivity-enhancing pathway is the traditional domain of the animal sciences. Collectively, we can help increase LWP by maximizing the value of animal products and services produced with available feed that is produced where transpiration is high and other forms of water depletion are low.

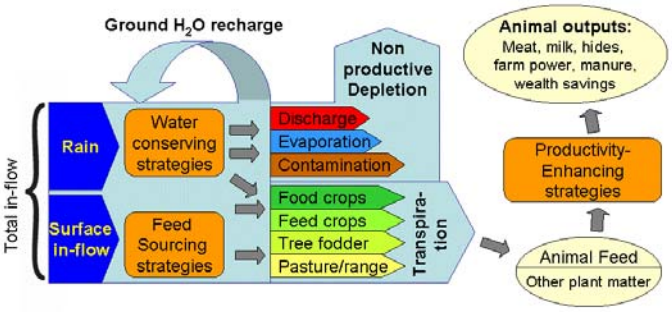


**Figure 1** Simplified assessment framework that helps identify strategies for improving livestock water productivity (Source: Peden 2007)

**Implications for Sub-Saharan Africa** Livestock production is an important part of African agriculture and animal densities are higher and lower respectively in irrigated and pastoral areas than in mixed crop-livestock systems. Africa is vulnerable to drought, waster scarcity and water-borne animal diseases including zoonotic ones. Increasing LWP through better management of livestock-water interactions holds promise for sustainably improving livelihoods of the continent's poor and making more fresh water available for other human needs and ecosystem services. Evidence from the CA (Peden 2007) indicates that investments in agricultural water development are often not sustainable and do not achieve potential returns on investments due to lack of integration of livestock. Contrary to much popular opinion, LWP compares favourably with marginal returns arising from investments in irrigated horticultural crops and is higher than observed in rain-fed grain crops. Water used for production of animal source food is currently the most effective means to meet protein, Vitamin B12, Iron and Selenium requirements of millions of malnourished Africans. The overarching message of the CA is that livestock-water interactions are important and under-researched and that huge opportunities exist to improve the productivity of water associated with livestock production. To achieve this will require active engagement of animal scientists in research and development of agricultural water in developing countries. Through an appropriate mix of technologies, management practices and policies, we estimate that current levels of animal production can be maintained while reducing water depletion by more than half in Sub-Saharan Africa.

**Acknowledgements** Funding and other support for this research was generously provided by the CGIAR *Comprehensive Assessment of transpiration Water Management and Agriculture*, the CGIAR *Challenge Program on Water and food* and the International livestock Research Institute.

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# Innovative *in vitro* and *ex vivo* models for studying bovine hoof biology

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**Introduction** Research into the biology and physiopathology of the bovine claw has become interdisciplinary employing epidemiology, cellular and molecular biology. Foot disorders in cattle are a global problem causing substantial economic losses to farmers. New hypotheses demonstrate possible links between systemic problems and local damage in the claw. Tissue explant and cell culture studies have already provided important insights into regulation of differentiation in healthy and diseased claw tissue (Hendry *et al* 1997, 2003; Nebel *et al* 2002, 2004). Detailed knowledge of the links between systemic events and the claw tissue as well as knowledge on the local regulatory cascades in response to metabolic and biomechanical challenges provide the key to understanding of the development of claw diseases. The development and experimental application of novel organotypic *in vitro* culture systems and an *ex vivo* isolated haemoperfused distal cow limb model (Wüstenberg 2004) was a major task and outcome of the EU framework 5 project Lamecow (http://www.abdn.ac.uk/lamecow).

**Material and Methods** Cell culture: Bovine keratinocytes and fibroblasts were isolated from claws of slaughtered cows and subsequently cultivated as described by Nebel and Mülling (2002). For perfusion culture fibroblasts were seeded onto the lower side of Millicell® inserts and allowed to grow for 4 days. Then the perfusion chamber was mounted by placing the fibroblast into the chamber and subsequently seeding epidermal keratinocytes onto the upper side of the insert allowing them to adhere for 1 day. Then the chamber was connected to the tube system with an attached Ismantec 8 channel pump (Isamantec, Wertheim-Mondfeld) and a gas permeable media bag (Oligene, Berlin). The system was run for up to 30 days. The generated organotypic tissue pieces were characterised by light and electron microscopy, immunocytochemistry and Western blotting. For studies on the regulation of cellular proliferation and differentiation immunohistochemistry for Il-1 and Il-1 receptor, KGF and KGF receptor were carried. RT-PCR was used to study regulation at the mRNA level in dermis and epidermis and in fibroblasts and keratinocytes from the cultures respectively. Isolated limb perfusion: Isolated distal cow forelimbs and autologous blood were obtained from routinely slaughtered healthy cows older than 24 months. Immediately after slaughter the 2 major digital arteries were cannulated and injected with an oxygenated electrolyte solution supplemented with glucose, heparin and insulin. Then the limb was transported to the laboratory at 4°C, where it was connected with the perfusion apparatus (Vitrotec Entwicklungs GmbH, Berlin, Germany). Arterial pressure, flow rate, organ resistance, $O_2$-saturation, oxy- and deoxygenated haemoglobin, pH, glucose were monitored. Tissue samples were obtained from 6 claw regions and processed for light and electron microscopy as well as cryo-stored for RNA-analysis by PCR. As criteria for vitality of the tissue we selected parameters of hemodynamics in combination with a light and electron microscopic evaluation of the tissue.

**Results** We developed a co-culture system of bovine hoof keratinocytes and fibroblasts in commercial available as well as newly developed prototype perfusion chambers. Our studies on the dermo epidermal cross-talk provide evidence for a local paracrine regulation of proliferation and differentiation of epidermal keratinocytes involving Il- 1 produced in the epidermis and KGF originating from fibroblasts. This mechanism is likely to be involved in response of claw epidermis upon mechanical overload or injury. Our challenge studies demonstrated that KGF and GM-CSF are potent stimulators of keratinocyte proliferation. TNF alpha, a central cytokine in ruminants had a depressive effect on keratinocyte proliferation and differentiation.
Isolated limb model: We developed and characterised an isolated haemoperfused distal cow limb model basing on a pre-existing porcine limb model (Wagner *et al* 2003), which was used for challenge experiments with bioactive molecules. A standard operation procedure (SOP) describes in detail the method and the equipment required for the limb perfusion. In a second phase we carried out a total of 52 perfusions challenging the model with nutrient deficiency, reduced oxygen and reduced blood flow, mediators/vasoactive substances and with histamine, lactate, endotoxine and vasoactive neuropeptides.

**Conclusions** The models presented here enable studies under standardised conditions from the cell to the organ level. Individual local regulatory cascades can be studied and hypotheses deriving from cell culture studies can then be tested in the limb model on the organ level. Our *in vitro* studies have demonstrated so far that the selected candidate molecules have effects that are relevant for the pathogenesis of non infectious claw diseases. The demonstrated effects *in vitro* will contribute to new hypothesis for the development of theses problems. They are promising molecular links between metabolic disorders in dairy cows and local tissue alterations in the claw leading to functional loss. Animal models are expensive to use and are invariably associated with concerns about animal welfare. *Ex vivo* isolated organ models provide the link between cell culture and animal experiments. They gain growing relevance in strategies to reduce and replace animal experiments.
In summary our results for the in vitro and ex vivo experiments strongly support the idea of complex rather than a simple physiopathology of laminitis and other claw diseases. Our results provide guidance for future research which should employ the model presented here to further explore the biology and physiopathology of the bovine hoof.

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# Measuring and preventing lameness in dairy cows

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**Introduction** In this presentation I review recent results from our research group on the assessment and prevention of lameness in dairy cows.

**Reliability and validity of lameness measures** Lameness can be defined as an abnormal gait due to pain in the hoof or leg. But how should gait be assessed? Much research to date has used subjective scoring systems that rely on a combined assessment of features including flatness of the back, bobbing of the head, the degree that the rear feet track-up with the fore feet, and perceived reluctance of the animal to place weight on any of the feet. More recently we have also used objective, kinematic measures of gait. We have assessed the degree to which these measures can be recorded reliably and if these are valid measures of the pain due to injuries.

We assessed the repeatability of the subjective gait assessments by comparing repeated measures of the same video sequences by the same and different observers (Flower and Weary, 2006), and have validated measures by comparing animals with and without injuries, receiving varying doses of analgesics, on soft and hard flooring (Flower et al., 2007), and after a period of treatment. Our overall gait score, and most of the component behaviours (e.g. back arch), could be scored reliably within and among observer. In all validation tests the overall gait score and two of the component measures (tracking up and reluctance to bear weight) performed well. The kinematic measures of gait were highly repeatable, but performed less well in most validation tests.

**Preventing lameness through improved barn design** Several studies have now shown that standing on concrete flooring is an important risk factor for hoof injuries leading to lameness, especially if the surface is wet. Increased standing times may be especially problematic in the days after calving.

We have studied how modifying indoor resting areas can promote lying and standing in the stall, and thus reduce standing on concrete surfaces elsewhere in the barn. Using a facility specifically designed for research on stall design, we have completed a series of experiments that have tested the effects of stall features using within-cow and within-pen experimental designs. Freestall surface is one of the most important features of the resting area; softer surfaces promote lying, such that rubber mats are better than concrete, mattresses better than mats, and bedded surfaces better than bare mattresses. Some stall surfaces, like deep-bedded sand or sawdust, can increase lying times by several hours a day and help prevent leg injuries such as hock lesions and swollen knees. Poor stall maintenance negates these advantages (Drissler et al., 2005), as does overstocking. Freestall design can also affect standing and lying times. Increasing stall width increases the time cows spend lying down and reduces standing outside of the stall (Tucker et al., 2004). Less restrictive neck-rail placement (higher and further from the rear of the stall) has little effect on lying times but reduces time spent standing outside of the stall (Tucker et al., 2005). Removing the brisket board increases lying time by over an hour a day, and reduces time spent standing outside of the stall (Tucker et al., 2006).

**Conclusions** A series of experiments has shown that both subjective scoring systems and objective kinematic measures can be used as valid indicators of lameness, but the subjective gait score showed the best performance in validation tests. Some but not all of the gait components used in scoring systems could also be scored reliably. Work on cow comfort has shown that well-maintained deep bedded stalls, with a minimum of restrictive hardware, increase the time that cows spend lying and standing in the stall, reducing the risk of lameness due to prolonged exposure to concrete standing areas.

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