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*ISBN 0 906562 39 2*

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# The effect of feeding intensity during the dry period on plasma leptin and time to return to cyclicity in dairy cows

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**Introduction** Much evidence has accumulated showing that female reproductive functions are disrupted when changes in nutritional status take place in both over- and undernutrition. The peptide hormone leptin is considered a possible link between nutrition and reproduction. One objective with the present study was to investigate how different levels of feed intake during the dry period, thereby creating differences in body condition at parturition, affected the plasma leptin concentration and the reproductive function after parturition.

**Material and methods** Multiparous dairy cows (n=24) were randomly allocated in three experimental groups. All groups were fed a total mixed ration (TMR) (0.45 silage, 0.15 hay and 0.40 concentrate on a dry matter (DM) basis) during the dry period (8 wk). The ration had the following composition: DM 640 g/kg, metabolisable energy (ME) 11.8/kg DM and amino acids absorbed in the small intestine (AAT) 7.4 g/MJ ME. The amount of MJ ME/day varied between the experimental groups (70MJ = Low; 105MJ = Medium; 170MJ = High). After parturition all cows were fed another TMR (0.40 silage, 0.05 hay and 0.55 concentrate on a DM basis) ad lib. for 12 weeks. This TMR had the following composition: DM 670 g/kg, ME 12.2/kg DM and AAT 7.5 g/MJ ME. Leptin was analysed in blood plasma 8 and 3 weeks before and 1, 3 and 12 week after parturition with a RIA-method evaluated for bovine leptin (Delavaud et al., 2000). Progesterone in milk was measured with a commercial RIA (Coat-A-Count, DCP Scandinavia) twice weekly from two weeks post parturition (p.p.) until 12 weeks p.p. in order to monitor the onset of cyclic activity and pinpoint cows with reproductive problems. Milk yield, body weight, and body condition scoring (BCS) were also registered. The General Linear Model of Minitab© was used for analysing effects of dry period treatment and of registration week respectively. Regression analysis between leptin and BCS and leptin and changes in BCS were also undertaken.

**Results** The plasma leptin concentration showed the highest value three weeks before parturition and the concentrations ranked according to intensity of the dry period feeding regimen (Table 1). Results from BCS are shown in Table 2. There was no difference in BCS eight weeks prior to parturition, before the animals were set on their different dry period treatments. One week after parturition there was a significant effect of treatment on the BCS. Twelve weeks after parturition the BCS had converged and there was no significant treatment effect.Leptin at three weeks prior to parturition correlated to the change in BCS during the dry period ($p<0.004$; $R^2=0.37$). The cows fed the low diet during the dry period gained 21 kg of body weight during the period from one week p.p. to twelve weeks p.p. On the other hand showed cows fed the high diet a reduction in body weight of 29 kg during the same period. The cows fed the medium diet during the dry period gained 1 kg of body weight. The effect of treatment was significant ($p<0.05$). The average milk yield during the first 12 weeks did not differ significantly between treatments. They yielded 38.6, (Low) 37.8 (Medium) and 38.5 (High) kg energy corrected milk (ECM)/day (pooled s.d.: 1.8 kg ECM/day). Cows fed the high intensity diet during the dry period had a long interval from parturition to normal cyclicity (51 days) according to the milk progesterone profiles. The corresponding values for the medium and low diets were 22 and 35 days respectively. The effect of treatment was significant ($p<0.05$; pooled s.d.: 8 days).

**Table 1** Plasma leptin (ng/ml)

| Week | -8 | -3[#] | 1 | 3 | 12 |
|---|---|---|---|---|---|
| Low | 4.1 | 4.6 | 3.3 | 3.6 | 3.9 |
| Medium | 4.7 | 5.6 | 3.0 | 4.7 | 3.9 |
| High | 4.9 | 7.3 | 3.8 | 4.2 | 4.5 |
| Pooled s.d. | 0.7 | 0.6 | 0.7 | 0.6 | 0.5 |

[#]Significant treatment effect ($p<0.05$)
Effect of week was significant ($p<0.001$)

**Table 2** Body Condition Score (Scale: 1-5).

| Week | -8 | 1[†] | 6 | 12 |
|---|---|---|---|---|
| Low | 3.6 | 2.6 | 3.0 | 2.8 |
| Medium | 3.6 | 3.6 | 3.4 | 3.4 |
| High | 3.8 | 4.2 | 3.9 | 3.6 |
| Pooled s.d | 0.4 | 0.7 | 0.3 | 0.7 |

[†]Significant treatment effect ($p<0.001$)

**Conclusions** The results from the present study confirm previous studies showing that cows fed to be overconditioned at parturition loose more body condition after parturition. These cows also showed a prolonged period from parturition to normal cyclicity which normally occurs about 25 days p.p. The cows fed the medium diet showed the shortest interval from parturition to cylicity. The leptin level three weeks prior to parturition was positively related to dry period feeding intensity and to gain of body condition. However, after parturition the level dropped in all three treatments and there was no difference between them. From these results it may be suggested that the range of postpartal concentrations of leptin observed in this trial did not have any significant impact on time to return to cyclicity.

**Reference** Delavaud, C., Bocquier, F., Chilliard, Y., Keisler DH., Gertler A., Kann G. 2000. Plasma leptin determination in ruminants: effect of nutritional status and body fatness on plasma leptin concentration assessed by a specific RIA in sheep. *Journal of Endocrinology*. **165**: 519-26.

# Relationship between plasma leptin concentration and reproductive function in dairy cows

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**Introduction** In modern dairy cows, poor fertility is an ever-increasing problem. Milk progesterone analysis has revealed that this poor fertility is associated with a high incidence of reproductive cycle problems during the post partum period and with reduced progesterone secretion following mating. One of the likely causes of these problems is the increasing metabolic demand placed on these cows by increasing milk yield. In recent years, the search for an endocrine link between nutritional status and reproductive function has focussed on leptin. The aim of the present study was to determine whether plasma concentrations of leptin differ between cows with and without post partum reproductive problems and to determine if any relationship exists between plasma leptin and post mating plasma progesterone concentration.

**Materials and methods** The study was carried out in 40 cows within the University of Nottingham commercial Holstein - Friesian herd calving during October and November. At 2, 6, 10 and 14 weeks post partum plasma samples were collected for leptin analysis by radioimmunoassay (Blache et al, 2000) and body condition score was recorded. Reproductive function was monitored by measuring progesterone by radioimmunoassay (Lamming and Bulman, 1976) in milk samples collected at weekly intervals throughout the study period. Milk progesterone profiles were used to determine onset of luteal activity (1$^{st}$ progesterone > 5ng/ml) and incidence of cycle problems (delayed onset of cyclicity - progesterone < 5ng/ml until > 65 days post partum; cessation of cyclicity - progesterone < 5ng/ml for > 2 weeks following period of > 5 ng/ml; luteal cysts - progesterone > 5ng/ml for >3 weeks. Throughout the study period, milk yield was recorded weekly. In addition, in 27 of the cows, additional plasma and milk samples were collected on day 5 following insemination to determine the adequacy of the post mating progesterone rise. These additional plasma samples were also assayed for leptin. Changes in plasma leptin and condition score over the experimental period were analysed by analysis of variance. Statistical comparisons between normal cows and cows with reproductive cycle problems were made using Student's t tests. Relationships between leptin, milk yield, condition score and onset of luteal activity were determined using linear regression analysis.

**Results** Condition score exhibited a significant decline from week 2 to week 6 (2.4±0.1 vs. 2.0±0.1; $p<0.001$) and then remained stable throughout the remaining study period. In contrast, plasma concentrations of leptin showed no significant change over the study period though there was a moderate correlation between mean plasma leptin concentration and mean condition score ($r = 0.49$; $p<0.005$). While leptin concentrations did not differ with time post partum, they did show considerable variation between cows, with mean concentrations ranging from 0.5 - 3.1 ng/ml. Plasma leptin concentration was not related to milk yield. Mean onset of luteal activity occurred on day 37.0±1.0 post partum and was not related to plasma leptin concentration or condition score but was correlated with milk yield ($r = 0.64$; $p<0.001$). Of the 40 cows studied, 20 had normal cycles and 20 exhibited reproductive cycle problems (6 delayed onset; 11 cessation; 3 luteal cysts). Cows exhibiting reproductive cycle problems had significantly lower plasma leptin than those with normal cycles (1.41±0.12 vs. 1.90±0.17 ng/ml; $p<0.05$). These cows with cycle problems also exhibited higher milk yield (32.7±1.8 vs. 28.2±0.9 l/d; $p<0.05$) and tended to have lower condition scores (1.8±0.1 vs. 2.0±0.1; $p=0.09$) than normal cows. For the 27 cows in which a plasma and milk sample were collected on day 5 after insemination there was a significant relationship between plasma leptin and both plasma ($r = 0.57$; $p<0.005$) and milk ($r = 0.63$; $p<0.005$) progesterone concentration. In these cows both plasma and milk progesterone concentrations were higher in cows conceiving to first insemination than in those returning to oestrus (plasma 2.3±0.3 vs. 1.8±0.1 ng/ml, $p<0.05$; milk 6.4±0.7 vs. 4.7±0.7 ng/ml, $p<0.05$). Although plasma leptin concentration was related to post ovulatory progesterone concentration, there was no significant difference in plasma leptin, either on day 5 post insemination or throughout the post partum period, between those cows conceiving to first service and those returning to oestrus.

**Conclusions** The results demonstrate that in lactating dairy cows both abnormal post partum reproductive cycles and poor post ovulatory progesterone secretion are associated with reduced plasma concentrations of leptin. Whether these lower leptin concentrations are a direct cause of poor reproductive function or the result of a common factor(s) such as poor metabolic status remains to be determined.

**Acknowledgements** This work was supported by the Ministry of Agriculture Fisheries and Food, the Milk Development Council and Intervet UK under the Link Sustainable Livestock Production Programme.

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Lamming, GE and Bulman, DC (1976). The use of milk progesterone radioimmunoassay in the diagnosis and treatment of subfertility in dairy cows. British Veterinary Journal 132, 507-517

Blache D, Tellam RL, Chagas LM, Blackberry MA, Vercoe PE and Martin GB (2000). Level of nutrition affects leptin concentrations in plasma and cerebrospinal fluid in sheep. Journal of Endocrinology, 165, 625 - 637.

# Leptin is involved in the regulation of ovarian activity in fasted hens (*Gallus domesticus)*

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**Introduction** In mammals leptin plays a key role in regulating the whole-body energy homeostasis and is important for for normal reproduction Much evidence indicates that leptin mediates the undernutrition-induced alterations of the reproductive axis (Ahima and Flier, 2000). In birds leptin gene was cloned in chickens (Taouis et al., 1998) and recombinant chicken leptin (chLep) was prepared in our laboratory (Raver et al., 1998). Unlike mammals, leptin is expressed not only in the adipose tissue but also in liver and leptin plasma level is lower in fasted than in fed hens (Dridi et al., 2000). However, the importance of leptin in avian reproduction is not known. Therefore, the aim of the present study was to examine whether in chickens leptin affects events occuring in the ovary during fasting.

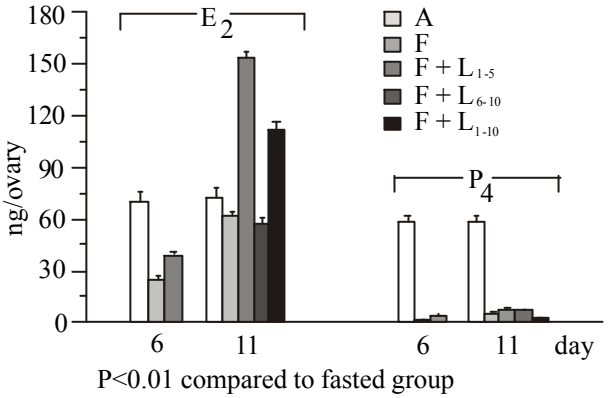
**Methods** Two experiments were performed on 112 Hy-line laying hens at 34 weeks of age. In both experiments hens were fasted from 1 to 5 day and then fed ad lib. Recombinant chLep (L) was injected (ip) 2 x daily (250 μg/kg) from 1 to 5 day ($L_{1-5}$) or 6 to 10 day ($L_{6-10}$) or 1 to 10 day ($L_{1-10}$). Exp. I: Hens (n=48) were divided in 4 equal groups: 1) fed ad lib. (A), 2) A + $L_{1-5}$, (3) fasted (F) and 4) F + $L_{1-5}$. Plasma LH, FSH, estradiol ($E_2$) and progesterone ($P_4$) levels were determined on days 0 and 6 and after return to egg lay. The ovaries were isolated on day 6 and after return to lay. Exp. II: Hens (n=64) were divided in 5 groups: 1) A (n=16), 2) F (n=16), 3) F+$L_{1-5}$ (n=16), 4) F+$L_{6-10}$ (n=8) and 5) F+$L_{1-10}$ (n=8). Plasma $E_2$ and $P_4$ levels were determined on days 0, 3, 6 and 11. Birds were slaughtered on days 6 and 11, the ovaries were isolated and $E_2$ and $P_4$ were determined in stroma and ovarian follicles. Results were analysed by two-way ANOVA, followed by the multiple range test of Duncan and statistical significance was inferred at $P<0.05$.

**Results** Exp. I: In fed ad libitum hens leptin had no effect on any of the examined parameters. In fasted hens on day 6 leptin: 1) delayed cessation of egg lay (Tab. 1), 2) significantly ($P<0.05$) attenuated the regression of the ovary, 3) had no effect on plasma LH and FSH levels and 4) significantly increased $E_2$ ($P<0.05$) and decreased $P_4$ ($P<0.01$) plasma levels. Exp. II: The changes of $E_2$ and $P_4$ content in the ovary are shown in Fig. 1. On day 6 in fasted hens leptin significantly: 1) increased $E_2$ level in blood and the ovary and 2) decreased $P_4$ level in blood but increased in the ovary. Most likely leptin hampered $P_4$ release to blood circulation as in comparison to fasted hens the morphology of hierarchical follicles was considerably changed in leptin treated hens. On day 11 the changes of plasma $E_2$ and $P_4$ levels and changes of their content in the ovary were parallel. In fasted hens: 1) leptin given for first 5 days significantly ($P<0.01$) increased $E_2$ content 2) prolongation of leptin injections to 10 days attenuated this effect, 3) leptin injected for 5 days after fasting had no effect on $E_2$ content and 4) the ovary weight and $P_4$ content was significantly ($P< 0.01$) decreased in hens treated with leptin for 10 days. Analysis of number and weight of ovarian follicles showed that prolongation of leptin administration to 10 days considerably diminished the selection of follicles into the hierarchy of yellow preovulatory follicles.

**Table 1** Effect of leptin on egg lay cessation

| group | Stop of egg lay (% hens) | | |
|---|---|---|---|
| | experiment day | | |
| | 3 | 4 | 5 |
| F | 58.33 | 94.44 | 100 |
| F + $L_{1-5}$ | 30.55 | 83.33 | 100 |

n = 36 for each group (exp. I + exp. II)




P<0.01 compared to fasted group

**Figure 1** Effect of leptin on $E_2$ and $P_4$ content in the ovary

**Conclusions** The results of the present study clearly show that in birds leptin plays the similar role as in mammals serving as a mediator for adaptation to starvation.

**Reference**

Ahima, R.S., Flier, J.S. 2000. Leptin. *Annual Reviews Physiology* **62:** 413-437.

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Dridi, S., Williams, J., Bruggeman, V., Onagbesan, M., Raver, N., Decuypere, E., Dijane, E., Gertler, A., Taouis, M. 2000. A chicken leptin-specific radioimmunoassay. *Domestic Animal Endocrinology* **18:** 325-335.

## Successful lactation in leptin-deficient obese (*ob/ob*) mice

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**Introduction** Mice lacking a functional leptin gene (*ob/ob*) are obese and sterile. Treatment with exogenous leptin will restore fertility and allow full-term pregnancy, but when leptin is withdrawn at parturition the young die, apparently as a result of total lactation failure (Chehab *et al.,* 1996). From this one could hypothesise that leptin is an essential requirement for mammary development and/or initiation of milk secretion. Since a few of the mice used in this work were subsequently able to rear pups following a second pregnancy and parturition, we decided to re-examine this hypothesis.

**Materials and methods** Male and female C57BL/6OlaHsd-***Lep***$^{ob}$ (*ob/ob*) and -lean (*lean*) mice were purchased from Harlan (Harlan UK, OX6 OTP U.K.), housed at a constant 17$^{O}$C on a 12/12h light/dark cycle and fed a standard laboratory chow (CRMX: BS&S, EH7 6UL, U.K.). Recombinant murine leptin was a generous gift of Dr A F Parlow (NHPP, CA 90509, USA). Other reagents were from Sigma (BH12 4OH, U.K.). In the first experiment groups of 6 *ob/ob* females were treated with cholesterol mixed with oestrogen and progesterone in the ratio 2002:1:1001 (Treated) or cholesterol alone (Control) as a solid 10mg pellet implanted s.c. under Halothane anaesthesia on d22 of life. Equivalent groups of 6 *lean* mice were treated identically. Fourteen days later all mice were killed and inguinal mammary glands were dissected and processed as whole mounts for histological counting of terminal end-buds (TEBs). In the second experiment 4 *ob/ob* females were treated by twice-daily injection with murine leptin (2.5 µg/g BW/d) commencing around d56 of life. After 7d they were placed singly with identically treated *ob/ob* males and examined daily for the presence of copulatory plugs. Leptin treatment was discontinued at mid-pregnancy and males were removed prior to parturition. Following parturition pups were removed and replaced by 6 new-born pups of a standard out-bred strain. Litter-swopping was repeated on days one and two of lactation. Pup growth rate and maternal food intakes were determined and compared with those of *lean* mice undergoing a normal first lactation. Statistical analysis was by Anova using Minitab Release 11 (Minitab, PA 16801-3008, USA).

**Results** Figure 1 shows TEB counts for treated and control mice. There were significantly more TEBs in *lean* (136.9±4.4) than in *ob/ob* (25.6±2.2). Treatment with oestrogen and progesterone increased TEBs significantly in both genotypes ($P<0.05$, Anova), and there was no significant interaction between treatment and genotype, indicating that the effect of treatment was equivalent in *lean* and *ob/ob*. Litter weight gains for *lean* and *ob/ob* mice are in Figure 2. The *ob/ob* mothers were unable to nourish young satisfactorily for the first 3d of lactation, thereafter weight gain increased gradually and by day 10 of lactation was indistinguishable from *lean.*

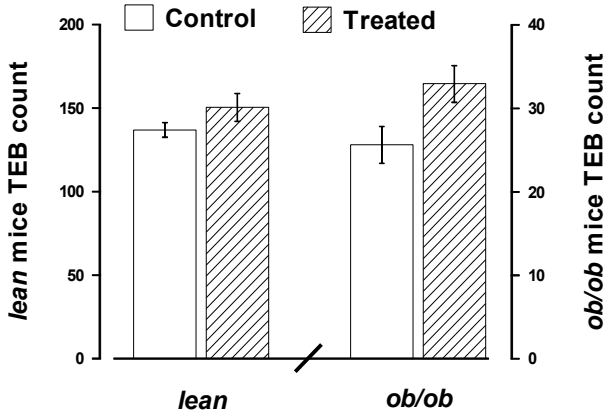


**Figure 1.** Terminal end bud counts

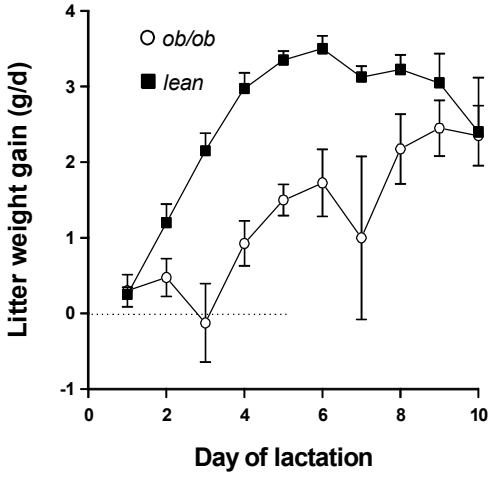


**Figure 2.** Litter weight gains

**Conclusions** There was a very definite impairment of prepubertal mammary development in *ob/ob* mice. Normal size was not restored by steroid treatment, nevertheless, the data show quite clearly that mammary tissue is capable of steroid-induced proliferation in the absence of leptin. The *ob/ob* defect is most likely due to a failure of endogenous steroid secretion rather than a direct consequence of leptin deficiency. Lactation was seriously compromised in the first few days *post partum* in *ob/ob* mice. However, once milk secretion was fully established it became quantitatively equivalent to *lean*, showing that there is no permanent defect of galactopoiesis. Further work will seek to establish whether the initial deficiency is due to inadequate mammary development, to a failure of lactogenesis or to inappropriate partitioning of nutrients between body reserves and mammary gland.

**References**

Chehab, F. F., Lim, M. E. and Lu, R. 1996. Correction of the sterility defect in homozygous obese female mice by treatment with the human recombinant leptin. *Nature Genetics* **12**: 318-320.

# Leptin is a signal of adiposity in fetuses of pregnant ewes fed at or above maintenance energy requirements

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**Introduction:** In the adult, circulating leptin concentrations are dependent on body fat content and on current nutrient intake. Whilst leptin concentrations in umbilical cord blood correlate with fetal adiposity in the human neonate, it is unknown whether leptin acts as a signal of fat mass before birth or whether changes in maternal nutrient intake alter plasma leptin concentrations in the fetus. We have therefore investigated the relationship between fetal plasma leptin concentrations and fetal fat mass in pregnant ewes fed at or above maintenance energy requirements.

**Materials and Methods:** Thirteen pregnant Border-Leicester Merino cross ewes were used in this study. Vascular catheters were inserted into each ewe and fetus at 103-112d gestation under general anesthesia. The experimental design was that pregnant ewes were randomly assigned at 115 days (d) gestation (term = 147 ± 3 d gestation) to either a Control group (n=5) that received a maintenance diet of 21.0 ± 2.0 g/kg of lucerne chaff (0.8 kg dry matter (DM)/kg; 8.3 MJ/kg DM) and 5.0 ± 0.5 g/kg of pelleted concentrate (0.9 kg DM/kg; 8.0 MJ/kg DM) daily, or to a Well fed group (n=8) that received 150-160% of maintenance requirements until 139-141 d gestation. At post mortem (140 ± 1d gestation) fetuses were delivered by hysterectomy and the weights of the interscapular and perirenal fat depots was recorded. To obtain a more sensitive measure of fat storage at each of these depots, quantitative histological analysis was used to calculate the volume density of unilocular adipose cells in both depots and the total (g) and relative (g/kg) mass of unilocular fat. Glucose and leptin concentrations were measured in maternal and fetal plasma using an enzymatic assay and competitive ELISA respectively. ANOVAs were used to assess the impact of an increase in maternal nutrient intake on maternal and fetal plasma concentrations of glucose and leptin and on fetal fat mass. Correlations between variables were determined using linear regression.

**Results:** Maternal plasma glucose and leptin concentrations were higher in Well fed ewes from 5 d after the start of the increase in maternal nutrient intake. Fetal plasma glucose concentrations were also higher in the Well-fed group (Control, 1.6 ± 0.1mmol/l; Well-fed, 2.0 ± 0.1 mmol/l; F=5.76, p<0.04). There was no effect of increased maternal nutrient intake on fetal plasma leptin (Figure 1) or total fat mass (Control, 22.8 ± 2.4 g; Well-fed, 23.9 ± 2.7 g ) or relative fat mass (Control, 5.1± 0.6 g/kg; Well-fed, 5.0 ± 0.5 g/kg). Fetal plasma leptin concentrations were not correlated with either glucose or insulin concentrations during late gestation. There was, however, a significant and positive relationship between fetal leptin concentrations at 139d gestation and the relative mass of unilocular fat in the fetal sheep when the Control and Well fed groups were combined (Figure 2).

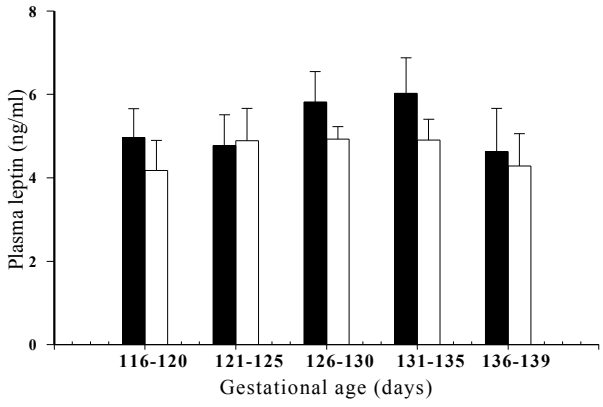


**Figure 1.** Effect of increased maternal nutrient intake on fetal plasma leptin concentrations in late gestation in Control (closed bars) and Well fed (open bars) groups.

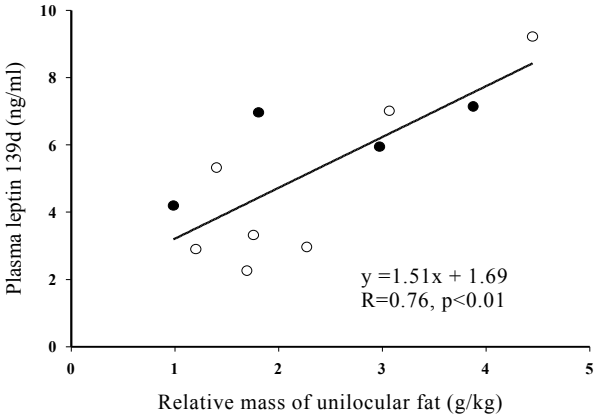


**Figure 2.** Correlation between fetal plasma leptin concentration at 139 d gestation (y) and relative mass of unilocular fat (x) in Control (closed circles) and Well fed (open circles) groups.

**Conclusions:** We have therefore demonstrated that fetal leptin acts as a signal of unilocular fat mass during late gestation in the sheep. There was no increase, however, in total or relative fetal fat mass or in circulating fetal leptin in ewes fed at 50-60% above energy maintenance requirements.

# Intrafetal infusion of leptin suppresses expression of leptin mRNA and increases the proportion of multilocular adipose tissue in fetal perirenal fat in the sheep

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**Introduction:** Leptin, a 16kDa polypeptide hormone, is synthesised and secreted by adipocytes and acts to regulate energy stores and energy expenditure in the adult. In the newborn infant, there is a positive correlation between cord blood leptin concentrations and neonatal fat mass but it is unknown whether leptin can act to regulate the endocrine or metabolic characteristics of adipose tissue before birth. We have therefore tested the hypothesis that an intrafetal infusion of leptin will act to alter on leptin mRNA expression and the morphological characteristics of perirenal fat, the major fat depot in the sheep fetus during late gestation.

**Materials and Methods:** Vascular catheters were inserted in 14 pregnant ewes and their fetuses at 110-124 days(d) gestation. AT 136/137 days gestation, fetal arterial blood samples (3ml) were collected at -3h, -2h, -1h and -30min relative to the start of the infusion protocol at 0h. Leptin (recombinant ovine leptin 0.5mg/ml (Kiesler et al)*:* 0.25mg bolus + 0.08mg/ 0.16ml/h iv) or saline (0.9% saline at 0.16ml/h) were infused for 96h via the fetal jugular vein and fetal arterial blood samples were then collected at +2min, +30min, +1h, +2h, +4h, +8h after the start of the infusion (infusion day 1) and at 0900h , 1300h and 1700h on both infusion day 2 and day 3 and at 0900h on infusion day 4. All fetal blood samples were centrifuged at 1500 g for 10 min and aliquots of plasma were separated and stored at -20ºC. Fetal plasma leptin concentrations were measured using a competitive ELISA. Fetal tissues were collected at 141 or 142 d gestation and leptin mRNA levels were measured in the fetal perirenal fat depot using RT-PCR.. The proportion of fetal perirenal fat which was comprised of either multilocular or unilocular tissue was measured using morphometric analysis.

**Results:** Intrafetal leptin infusion significantly increased fetal plasma leptin concentrations throughout the infusion period (Leptin infused, 15.8 ±3.3 ng/ml; Saline infused, 3.9± 0.6 ng/ml). The relative abundance of leptin mRNA : β-actin in perirenal adipose tissue was suppressed in leptin infused fetuses (Leptin infused, 0.39 ± 0.02; Saline infused, 0.45 ± 0.01). Leptin infusion also resulted in a change in the relative proportions of unilocular and multilocular fat in the fetal perirenal adipose tissue (Table 1). There was no effect, however, of intrafetal leptin infusion on either the total or relative mass of fetal perirenal fat.

**Table 1: Proportion of Unilocular and Multilocular fat in Leptin and Saline Infused Fetal Sheep**

| | **Leptin Infused Group** | **Saline Infused Group** |
|---|---|---|
| **Unilocular fat (%)** | 31.6 ± 2.3% | 44.7 ± 4.0% |
| **Multilocular fat (%)** | 61.6±2.0% | 48.7±3.4%. |

**Conclusions:** We have demonstrated for the first time that an increase in circulating leptin concentrations results in a change in the potential leptin synthetic capacity and the morphological characteristics of fetal adipose tissue. These results indicate that leptin may act to regulate aspects of energy balance before birth.

# The effect of long-chain polyunsaturated fatty acid and vitamin E supplementation of ewes on neonatal lamb vigour, lamb growth and colostrum parameters

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**Introduction** The long-chain polyunsaturated fatty acids (PUFA) docosahexaenoic acid (DHA) and arachidonic acid (AA) are the most abundant fatty acids in the brain and are vital for its correct development and for that of the nervous system (Huang and Craig-Schmidt, 1996). Ruminant diets are low in DHA and its precursor alpha-linolenic acid. In addition, dietary PUFAs are substantially hydrogenated in the rumen. Consequently, it may be argued that the diets of pregnant and lactating ewes may be deficient in DHA and that a response to supplementation may be observed. Studies involving the supplementation of pregnant ewes with supraoptimal levels of vitamin E have shown that lambs born to supplemented dams are more vigorous immediately after birth and have higher liveweight gains (Merrell, 1998). The objective of this experiment was to investigate the effects of dietary long-chain PUFA in combination with vitamin E supplementation of ewes on ewe and lamb performance.

**Materials and methods** Forty eight Lleyn and Mule ewes were allocated to one of four treatments at 103 days of gestation and blocked according to age, breed, litter size (two or three lambs), liveweight and condition score in a 2 x 2 factorial, randomised block design. Ewes were individually penned and housed from six weeks *pre-partum* to four weeks *post partum* and fed one of four treatment concentrates, each containing either fish oil plus Incromega (a source of docosahexaenoic acid fed at a 25:75 ratio with fish oil) or Megalac™ (control) as the main fat source and a basal (50mg/kg) or supraoptimal (500mg/kg) concentration of vitamin E. The concentrates were isoenergetic, isonitrogenous and formulated to provide 80g fat/kg DM. Straw was offered *ad-libitum*. At lambing, lambs were focal sampled and the latencies of standing (lamb supporting itself on all four feet) and successful suckling (lamb has the teat in its mouth and appears to suck) were recorded. To avoid confounding behavioural observations, birthweight data was recorded at +12 hours *post partum*, with subsequent weighings at 7, 14, 21 and 28 days of age. Ewes were milked at +12 hours and +16 hours *post partum* to provide samples for fatty acid analysis and to provide a measure of colostrum yield. Colostrum fatty acid analysis was performed by gas chromatography. Data was analysed by ANOVA.

**Results** The length of gestation was significantly increased in ewes fed fish/DHA (s.e.d 0.51, $P<0.001$; Table 1) and lambs born to these ewes suckled, on average, 9.4 minutes sooner than lambs born to ewes fed diets containing Megalac™ (s.e.d 3.48, $P<0.01$). In addition, supraoptimal vitamin E supplementation significantly reduced the latency of standing in lambs from ewes fed fish/DHA compared to ewes fed Megalac™ ($P<0.05$). Supplementation of pregnant ewes with supraoptimal levels of vitamin E resulted in a 0.23kg increase in lamb birthweight compared to supplementation with a basal level of vitamin E (s.e.d 0.268, $P<0.05$). Lamb growth rate from birth to four weeks of age was significantly reduced in lambs from ewes fed fish/DHA compared to Megalac™ (0.24kg/day *v*. 0.27kg/day, s.e.d 0.011, $P<0.01$). Ewes offered diets containing fish/DHA had a significantly lower colostrum yield compared to that of ewes fed diets containing Megalac™ (1.79l/day *v*. 2.56l/day, s.e.d 0.257, $P<0.01$). Colostrum fat concentration was also significantly depressed by the supplementation of ewes with fish/DHA (102.7g/kg *v*. 128.5g/kg, s.e.d 6.16, $P<0.001$), however, the DHA concentration was greater in colostrum from ewes fed fish/DHA ($P<0.001$).

**Table 1** Effects of supplementing ewes with long-chain polyunsaturated fatty acids and vitamin E on neonatal lamb vigour, lamb growth and colostrum parameters

| | Treatment Diet[a] | | | | | Significance | | |
|---|---|---|---|---|---|---|---|---|
| | MB | MS | FB | FS | s.e.d | Fish | Vitamin E | Interaction |
| Gestation Length | 145.2 | 145.8 | 148.3 | 147.1 | 0.72 | *** | NS | NS |
| Latency to stand (min) | 17.0 | 23.6 | 17.6 | 14.9 | 3.23 | NS | NS | * |
| Latency to suckle successfully (min) | 38.2 | 48.7 | 33.6 | 34.5 | 4.92 | ** | NS | NS |
| Lamb birthweight (kg) | 3.87 | 4.01 | 3.85 | 4.33 | 0.190 | NS | * | NS |
| Lamb growth rate (kg/day) | 0.27 | 0.28 | 0.24 | 0.24 | 0.011 | ** | NS | NS |
| Colostrum yield (l/day) | 2.27 | 2.85 | 1.86 | 1.73 | 0.364 | ** | NS | NS |
| Colostrum fat (g/kg) | 122.5 | 134.6 | 101.1 | 104.3 | 8.71 | *** | NS | NS |
| Colostrum DHA (g/100g fat) | 0.13 | 0.00 | 0.51 | 0.52 | 0.056 | *** | NS | NS |

[a] MB = Megalac™ + 50mg/kg vitamin E; MS = Megalac™ + 500mg/kg vitamin E; FB = Fish/DHA + 50mg/kg vitamin E; FS = Fish/DHA + 500mg/kg vitamin E

**Conclusions** Long-chain PUFA supplementation of ewes had beneficial effects on lamb vigour, however, it resulted in lower colostrum fat and yield and consequently reduced growth rate. Supraoptimal vitamin E supplementation of ewes significantly increased lamb birthweight, however it did not directly affect lamb behaviour.

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## Influence of dietary fatty acids on the fatty acid composition of mesenteric lymph nodes (MLN) and spleen in the milk fed calf.

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**Introduction:** Dietary fatty acids have been shown to affect the activity of the immune system in a variety of species through eicosanoid dependent or independent mechanisms (Miles 1998, Calder 2001). MLN and spleen are lymphoid tissues which play a key role in the immune function. Changing the fatty acid composition of these tissues could change the profile of eicosanoid produced by immune cells in these tissues and this could alter the immune response. This study was carried out to establish the extent to which different oil supplements could change the fatty acid composition of MLN and spleen in milk fed calves.

**Materials and Methods:** Twenty-four pre-ruminant calves, were allocated, according to age, to two treatment groups (n=12) which received, as a supplement to milk replacer, 25g/d of either an n-3 PUFA-rich fish oil (n-3) or a binary mixture of palm oil and rape seed oil (normal) designed to supply the same fatty acid profile as found in milk replacer. α-Tocopherol acetate (30mg /kg PUFA) was added to the milk replacer. All the calves were also provided with weaner pellets and hay. Total nutrient intake was calculated to provide 1.5 x maintenance energy requirements. Calves were killed 9 weeks post supplementation, after being fasted for 24 hours. Lipids from mesenteric lymph nodes and spleen were extracted using the 'Folch' procedure and the fatty acid methyl esters analysed by gas-liquid chromatography. Data were analysed by one-way ANOVA.

**Results:** In MLN and spleen the major changes in individual fatty acids were significant increases in the concentrations of C20:5n-3, C22:5n-3, and C22:6n-3 in the n-3 supplemented group. As a result there was a significant decrease in the n6/n3 ratio in the group. Supplementation with fish oil had no significant effects on total saturated (SFA), monounsaturated (MUFA), and polyunsaturated fatty acids (PUFA).

**Table 1**. Comparison of fatty acid composition of lipid from mesenteric lymph node and spleen (g fatty acid/kg total fatty acids).

| Mesenteric lymph nodes | | | | Spleen | | |
|---|---|---|---|---|---|---|
| Fatty acid | n-3 group | Normal group | significance | n-3 group | Normal group | significance |
| C14:0 | 34.4 ± 3.0 | 34.7 ± 1.0 | N/S | 35.9 ± 6.6 | 36.2 ± 5.9 | N/S |
| C16:0 | 239.8 ± 3.5 | 229.8 ± 1.8 | N/S | 241.9 ± 5.9 | 241.5 ± 7.3 | N/S |
| C18:0 | 193.4 ± 4.7 | 189.2 ± 3.3 | N/S | 197.2 ± 4.5 | 203.9 ± 3.9 | N/S |
| C18:1n-9 | 268.7 ± 3.8 | 304.7 ± 8.2 | N/S | 165.8 ± 5.4 | 177.5 ± 6.8 | N/S |
| C18:2n-6 | 65.6 ± 1.0 | 76.9 ± 1.7 | ** | 58.7 ± 2.6 | 63.7 ± 2.1 | N/S |
| C18:3n-3 | 5.3 ± 0.8 | 7.4 ± 1.2 | N/S | 2.2 ± 0.7 | 1.8 ± 0.3 | N/S |
| C20:0 | 2.8 ± 0.1 | 2.8 ± 0.2 | N/S | 3.0 ± 0.2 | 4.2 ± 0.5 | N/S |
| C20: 1n-11 | 6.7 ± 0.5 | 5.8 ± 0.1 | N/S | 6.4 ± 0.8 | 9.2 ± 0.6 | N/S |
| C20:2n-6 | 1.7 ± 0.1 | 2.3 ± 0.2 | ** | 6.4 ± 0.8 | 10.8 ± 1.2 | ** |
| C20:4n-6 | 26.4 ± 2.9 | 30.4 ± 3.2 | N/S | 55.2 ± 3.7 | 63.4 ± 8.5 | N/S |
| C20:5n-3 | 13.7 ± 2.4 | 2.9 ± 0.3 | *** | 20.6 ± 2.4 | 4.7 ± 0.7 | *** |
| C22:5n-3 | 25.0 ± 2.8 | 15.6 ± 1.9 | *** | 36.9 ± 2.6 | 19. 0 ± 2.1 | *** |
| C22:6n-3 | 12.1 ± 1.2 | 4.1 ± 0.4 | *** | 18.0 ± 1.5 | 4.9 ± 1.0 | *** |
| ΣSFAs | 497.3 ± 7.7 | 482.5 ± 4.2 | N/S | 523.3 ± 6.3 | 527.2 ± 9.1 | N/S |
| ΣMUFAs | 295.7 ± 13.2 | 322.9 ± 4.2 | N/S | 178.8 ± 5.3 | 192.4 ± 7.4 | N/S |
| ΣPUFAs | 157.0 ± 9.5 | 145.0 ± 5.3 | N/S | 213.3 ± 7.3 | 192.2 ± 8.7 | N/S |
| P/S ratio | 0.3 ± 0.2 | 0.3 ± 0.1 | N/S | 0.41 ± 0.1 | 0.37 ± 0.02 | *** |
| n-6/n-3 ratio | 0.3 ± 0.1 | 0.6 ± 0.1 | *** | 0.23 ± 0.1 | 0.6 ± 0.02 | *** |

NS = not significant, ** p=<0.01; *** = p<0.001;

**Conclusions**. In calves, milk is a suitable medium for oil supplementation when the oesophageal groove reflex is maintained. Fish oil in the n-3 group clearly influenced the fatty acid composition of mesenteric lymph nodes and spleen within a period of 9 weeks compared to a binary mixture of palm and rapesseed oil in the normal group. The decrease in C20: 4n-6/ C20: 5n-3 and n-6/n-3 ratio in the n-3 group may have consequences on the relative cellular eocosanoids synthesis and thus the development and activity of the immune system.

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# The effects of including ruminally protected lipid in the diet of Charolais steers on animal performance, carcass quality and the fatty acid composition of longissimus dorsi muscle

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**Introduction** Increasing the ratio polyunsaturated:saturated fatty acids (P:S) in beef muscle by nutrition is hampered by the high levels of ruminal biohydrogenation of dietary polyunsaturated fatty acids (PUFA). Effective ruminal protection of dietary fatty acids, such as that provided by encapsulation of PUFA in formaldehyde-treated protein may ameliorate this situation. This study evaluated the effects of including in the diet a ruminally protected lipid supplement (PLS), containing linoleic (C18:2) and *a*-linolenic (C18:3) acids, on the fatty acid composition of the m. *longissimus*.

**Materials and methods** Twenty four Charolais steers (initial live weight 528 (s.e. 6.3) kg) were randomly allocated to one of three dietary treatments, each consisting of eight animals. The diets were based on *ad libitum* grass silage plus one of three concentrates in which the lipid source was either Megalac (Mega, rich in palmitic acid; C16:0) or PLS (soya beans, linseed and sunflower oils resulting in a 2:1 ratio of C18:2 n-6 : C18:3 n-3): Concentrate 1, (Mega, control) contained 100g/kg Mega; Concentrate 2, (Mega + PLS) contained 54g/kg Mega with 500 g/d PLS fed separately; Concentrate 3, (PLS) contained no Mega and 1000 g/d PLS fed separately. The PLS was considered as part of the overall concentrate allocation per day in maintaining an overall forage:concentrate ratio of 60:40 on a DM basis. To ensure that total dietary oil and protein intake was balanced across the 3 treatment groups, the 3 concentrates differed in formulation and hence chemical composition. The TN and AHEE were 23.5, 20.6 and 17.4 and 108, 65 and 19 g/kg DM for Mega, Mega + PLS and PLS concentrates, respectively. The TN and AHEE of the protected lipid supplement was 48.0 and 366 g/kg DM, respectively. Total dietary oil was formulated to be 0.07 of DM of which 0.04 was the test oil. Daily feed intakes and live weights were monitored. Animals were slaughtered after 90 days on treatment, carcasses were classified and samples of *longissimus dorsi* were acquired for fatty acid analysis. An ANOVA with diet as the main factor was used to analyse the animal performance, carcass and fatty acid results.

**Results** Total DM intake, liveweight gain, carcass weight and fat score (0-100 scale) were similar across treatments and averaged 9.88 kg/d (s.e.d. 0.338), 1.4 kg/d (s.e.d 0.10), 359 kg (s.e.d. 8.5) 83.1 (s.e.d. 7.30), respectively. Total fatty acids were decreased (by 0.31) when feeding PLS compared to Mega ($P < 0.05$; Table 1). The saturated fatty acids, C14:0, C16:0 and C18:0 were reduced by feeding Mega + PLS and PLS and were on average 0.38 lower on PLS compared to the Mega diet. Similarly, oleic acid, C18:1 n-9 ($P < 0.05$) on PLS diet and C18:1 *trans* ($P < 0.002$) was lower on Mega + PLS and PLS. On average, feeding PLS doubled the content of C18:2 n-6 and C18:3 n-3. CLA content decreased with inclusion of PLS in line with the reduction in total fatty acids. Both the content and percentage of the longer chain derivatives of C18:2 n-6, for example C20:4 n-6, were not changed by feeding PLS. The content of C20:5 n-3 (EPA; synthesised from C18:3 n-3), was not affected by PLS, but the percentage was increased, on average by 0.15 and 0.42 by feeding Mega + PLS and PLS respectively, compared to Mega. The P:S ratio was increased with inclusion of PLS ($P < 0.001$).

**Table 1** Fatty acid content (mg/100g muscle) of m.longissimus dorsi

| | Mega | Mega + PLS | PLS | s.e.d. | P |
|---|---|---|---|---|---|
| C14:0 myristic | 107.5 | 85.1 | 64.5 | 15.24 | 0.034 |
| C16:0 palmitic | 986 | 843 | 598 | 117.8 | 0.012 |
| C18:0 stearic | 508 | 421 | 331 | 61.6 | 0.03 |
| C18:1 *trans* | 73.9 | 53 | 38.1 | 8.47 | 0.002 |
| C18:1n-9 oleic | 1195 | 1144 | 759 | 177 | 0.044 |
| C18:1 cis vaccenic | 38.2 | 37 | 26.9 | 4.81 | 0.053 |
| C18:2 n-6 linoleic | 100.3 | 194.6 | 215.1 | 9.46 | 0.001 |
| C18:3 n-3 α-linolenic | 23.1 | 45.9 | 45.8 | 4.25 | 0.001 |
| CLA cis-9, trans-11 C18:2 | 16.6 | 14.4 | 9.9 | 2.56 | 0.044 |
| C20:3 n-6 | 9.53 | 10.23 | 8.66 | 0.656 | 0.081 |
| C20:4 n-6 arachidonic | 28.2 | 27.2 | 28.3 | 2.02 | NS |
| C20:5 n-3 eicosapentaenoic (EPA) | 9.52 | 9.51 | 8.93 | 1.198 | NS |
| C22:5 n-3 docosapentaenoic (DPA) | 18.8 | 16.3 | 14.7 | 1.13 | 0.005 |
| C22:6 n-3 docosahexaenoic (DHA) | 2.25 | 1.66 | 1.72 | 0.355 | NS |
| Total fatty acids | 3505 | 3260 | 2421 | 430.8 | 0.049 |
| P:S ratio | 0.06 | 0.19 | 0.28 | 0.029 | 0.001 |

**Conclusions** The results suggest that the protected lipid used, which was rich in PUFA, had a high degree of protection from the hydrogenating action of rumen microorganisms, resulting in substantial reductions in saturated fatty acids while PUFA were increased, resulting in a large shift in the P:S ratio towards 'healthier' beef.

**Acknowledgements** This work was supported by UK Ministry of Agriculture Fisheries and Food, ABN Limited, Tesco Stores Limited, Pedigree Petfoods, Rarebreeds Survival Trust and Roche Products Limited.

# Biohydrogenation and duodenal flow of C18 polyunsaturated fatty acids in steers fed grass or grass : legume silages.

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**Introduction** Forages are a rich source of beneficial *n-3* PUFAs (?-linolenic acid; C18:*3n*-3) and may be a useful means of modifying the fatty acid composition of ruminant products. Altering the fatty acid composition of ruminant products is difficult due to the biohydrogenation action of rumen micro-organisms, which hydrogenate the PUFAs into monounsaturated and saturated fatty acids. Little information is available on biohydrogenation of PUFAs in forages hence this study investigated this in steers fed on perennial ryegrass, red or white clover silages or combinations thereof.

**Materials and methods** Ten Hereford x Friesian steers 362 (se 7.7) kg, prepared with rumen and duodenal cannulae were allocated at random to receive one of five silage diets *ad libitum;* grass (G); grass and white clover (60:40 DM basis; GW); grass and red clover (60:40 DM basis; GR); white clover (W) and red clover (R). The experiment was conducted as a two period changeover design within each legume treatment, with grass silage as an experimental control. Animals allocated to red clover or white clover remained on the same legume treatment but the percentage of legume changed across the periods. Animals allocated to grass silage remained on that feed in both periods. Each period lasted 24 days, with a 14 day adaptation period to the diets, followed by a 10 day measurement period. Statistical analysis was undertaken using REML, with silage as the fixed effect and animal + period × period as the random effect (Genstat 5; Lawes Agricultural trust, 1997).

**Results** The chemical and fatty acid composition of the silages were significantly different for most of the components measured. The legume silages (W and R) had higher levels of water-soluble carbohydrate, starch and total nitrogen and lower concentrations of fibre, ether extract and ammonia nitrogen than the grass silage (G) ($P<0.001$) with the two grass : legume silages (GW and GR) an intermediate. The total fatty acid content of W (25.6 g/kg DM) was greater ($P<0.001$) than the other silage diets and that of R and GW (21.1 and 19.5 g/kg DM, respectively) greater than GR and G (16.0 and 18.0 g/kg DM, respectively). Rumen parameters were not significantly different across treatments. The DM intake of the silages, individual fatty acid intake and individual fatty acid flows at the duodenum of the C18 fatty acids in animals fed on the five silage diets were significantly different (Table 1). Rumenic and vaccenic (c) acid flows at the duodenum were significantly higher in animals on the two white clover silages W and GW than animals on the other silage diets ($P=0.005$). Both linoleic and linolenic acid duodenal flows were higher in animals on the legume silages than those on the grass silage. Biohydrogenation of linolenic acid was significantly lower on the red clover silage diets R and GR.

**Table 1**. Effect of forage type on intake and ruminal fatty acid metabolism.

| | G | R | GR | W | GW | s.e.d | P |
|---|---|---|---|---|---|---|---|
| DM Intake (kg/d) | 4.2 | 7.0 | 6.4 | 8.5 | 8.4 | 0.64 | 0.027 |
| Fatty acid intake (g/day) | | | | | | | |
| C18:0 stearic | 1.53 | 4.50 | 2.60 | 5.90 | 4.07 | 0.391 | 0.001 |
| C18:2*n-6* linoleic | 11.2 | 26.8 | 18.0 | 41.5 | 30.5 | 2.63 | 0.001 |
| C18:3*n-3* linolenic | 39.1 | 73.8 | 49.4 | 111.1 | 83.0 | 7.27 | 0.001 |
| Total fatty acids | 74.7 | 147.9 | 101.9 | 217.0 | 164.7 | 14.33 | 0.001 |
| Fatty acid flow to duodenum (g/day) | | | | | | | |
| C18:0 stearic | 61.6 | 80.9 | 70.5 | 103.7 | 77.0 | 18.35 | NS |
| C18:2*c9t11* rumenic (CLA) | 0.74 | 1.53 | 0.97 | 2.66 | 1.87 | 0.494 | 0.005 |
| C18:2*n-6* linoleic | 1.44 | 4.72 | 4.25 | 6.60 | 4.95 | 1.400 | 0.014 |
| C18:3*n-3* linolenic | 2.70 | 11.8 | 13.7 | 11.4 | 9.5 | 2.84 | 0.017 |
| Total fatty acids | 109.0 | 155.0 | 155.0 | 193.0 | 163.0 | 38.60 | NS |
| Biohydrogenation (%) | | | | | | | |
| C18:2*n-6* linoleic | 87 | 83 | 77 | 85 | 83 | 4.3 | NS |
| C18:3*n-3* linolenic | 93 | 84 | 73 | 90 | 88 | 3.5 | 0.001 |

**Conclusion** The white clover silage diets both significantly elevated the duodenal flow of rumenic and vaccenic (c) acids. The legume silages appeared to elevate the duodenal flow of both linoleic and linolenic acids but this may have been a response to the greater intake of these acids, through a greater fatty acid concentration in the clover and/or the greater DM intake. The reduction in biohydrogenation of linolenic acid on the red clover silages may have also increased its flow at the duodenum. These results help to explain the higher content of *n-6* and *n-3* PUFAs in beef muscle of animals produced on grass and legume systems (Enser *et al*. 2001).

**Acknowledgements** This study was supported by DEFRA and the European Commission.

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# Influence of dietary *n*-3 polyunsaturated fatty acids on milk fat composition and performance of lactating Friesland ewes

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**Introduction** The health benefits of *n*-3 polyunsaturated fatty acids (PUFA) and conjugated linoleic acid (CLA) to humans are now widely recognised. Polyunsaturated fatty acids of the *n*-3 series such as α-linolenic acid (C18:3*n*-3), eicosapentaenoic acid (C20:5*n*-3; EPA) and docosahexaenoic acid (C22:6*n*-3; DHA) reduce the risk of coronary heart diseases (Department of Health, 1994). CLA has a diverse array of potential beneficial health effects which include anticarcinogenesis, antiatherogenesis, immune system modulation, antidiabetic effects and reduction of body fat accretion (Bessa et al., 2000). However, the consumption of both *n*-3 PUFA and CLA by humans is currently less than optimal. The current study investigates the extent to which *n*-3 PUFA and CLA in milk fat of lactating ewes may be enhanced by feeding diets rich in EPA and DHA.

**Materials and Methods** Twenty multiparous Friesland ewes (74 ±7.8kg weight) in week four of lactation were grouped into five groups which were balanced with respect to milk yield, and randomly assigned to five diets, over four 28-day periods, in a replicated incomplete 5 × 4 Latin Square design. On a fresh weight basis, diets contained (g/kg) 350 grass hay, 330 barley, 115 soya bean meal, 110 rape seed meal, 60 molasses, 30 minerals plus vitamins, and 5 urea. Diets were formulated to contain 70g fat/kg dry matter (DM) by replacing part of the barley in the basal diet with five lipid supplements namely; Megalac (Control), Fish oil, FOV [fish oil + vermiculite, 50:50 (wt/wt)], Omega-3 (prilled, fish oil + linseed oil) and Incromega (fish oil derivative). The ewes had *ad libitum* access to feed and water, and were milked twice daily (0730 and 1500 h). Milk samples (a.m. and p.m.) were collected over 72 hours at the end of the final week of each period. DM intake, milk yield and concentrations of major milk constituents were determined. The fatty acid profiles of milk fat were analysed by gas chromatography. Analysis of variance was done using Genstat 5.

**Results** Relative to the Control, DM intake was significantly reduced by all fat supplements ($P < 0.001$), with the lowest intake occurring with the fish oil diet (Table 1). Ewes fed either Omega-3 or Incromega produced significantly greater amounts of milk compared to those on Megalac, whilst supplying either of the two fish oil treatments induced a significant reduction ($P < 0.001$). Compared with the Megalac treatment, provision of any of the four supplements caused milk fat depression ($P < 0.001$), with the greatest decline being experienced in ewes fed either Fish oil or Incromega. All supplements with the exception of Omega-3, caused a significant decrease in milk protein concentration compared to the Control ($P < 0.001$). CLA levels were increased by 3 to 5 times by the lipid supplements compared to the Control ($P < 0.001$). This effect was most pronounced when Omega-3 was fed. The concentration of C18:3*n*-3 in milk fat was enhanced by the four treatments ($P < 0.001$), with highest values being recorded when Omega-3 was fed. Provision of any of the test supplements induced a 6- to 8-fold increase in the concentration of C20:5*n*-3 relative to Megalac ($P < 0.001$), with the response declining in the following sequence: Incromega > Omega-3 > Fish oil > FOV. Compared to the Control, C22:6*n*-3 increased by 4.0, 5.1, 9.5 and 9.9 times when Fish oil, FOV, Omega-3 and Incromega were fed respectively ($P < 0.001$). Ingested EPA was transferred into milk fat at efficiencies ranging from 2.0% with Fish oil to 6.6% with Omega-3, and DHA at 2.2% with Fish oil to 5.3% with Omega-3.

**Table 1:** Effects of different dietary *n*-3 PUFA sources on the performance of lactating ewes and milk fat composition

| | Treatment | | | | | | |
|---|---|---|---|---|---|---|---|
| | Megalac | Fish oil | FOV | Omega-3 | Incromega | *s.e.d.* | *F-Probability* |
| DM intake (kg/d) | 3.04$^{a}$ | 2.27$^{b}$ | 2.54$^{c}$ | 2.80$^{d}$ | 2.88$^{d}$ | 0.077 | $P < 0.001$ |
| Milk yield (g/d) | 1931$^{a}$ | 1737$^{b}$ | 1852$^{b}$ | 2106$^{c}$ | 2148$^{c}$ | 82.7 | $P < 0.001$ |
| Milk fat (g/kg) | 60.0$^{a}$ | 37.8$^{b}$ | 41.6$^{c}$ | 40.0$^{c}$ | 35.6$^{b}$ | 1.70 | $P < 0.001$ |
| Milk protein (g/kg) | 44.8$^{a}$ | 42.6$^{b}$ | 42.4$^{b}$ | 43.5$^{ab}$ | 42.8$^{b}$ | 0.86 | $P < 0.001$ |
| Milk fatty acids (g/100g) | | | | | | | |
| C18:2 *n*-6 | 3.1$^{a}$ | 2.3$^{b}$ | 2.0$^{b}$ | 1.8$^{b}$ | 2.2$^{b}$ | 0.24 | $P < 0.001$ |
| CLA | 0.56$^{a}$ | 1.82$^{b}$ | 2.62$^{cd}$ | 2.93$^{c}$ | 2.41$^{d}$ | 0.185 | $P < 0.001$ |
| C18:3 *n*-3 | 0.39$^{a}$ | 0.56$^{be}$ | 0.51$^{c}$ | 0.64$^{d}$ | 0.58$^{e}$ | 0.031 | $P < 0.001$ |
| C20:5 *n*-3 | 0.10$^{a}$ | 0.62$^{b}$ | 0.60$^{b}$ | 0.69$^{b}$ | 0.84$^{c}$ | 0.051 | $P < 0.001$ |
| C22:6 *n*-3 | 0.08$^{a}$ | 0.32$^{b}$ | 0.41$^{b}$ | 0.76$^{c}$ | 0.79$^{c}$ | 0.047 | $P < 0.001$ |

**Conclusions** The profile of milk fatty acids can be manipulated to increase the content of nutritionally beneficial *n*-3 PUFA and CLA by dietary supplementation of lactating ewe diets with either fish oil or its derivatives. Nevertheless, the transfer efficiencies of ingested *n*-3 PUFA into milk fat are low. Vermiculite treatment of fish oil was not effective in enhancing the incorporation of *n*-3 PUFA into milk fat, but it did increase DM intake compared to fish oil fed alone.

**Acknowledgements** We are grateful to Mr John Twigge (Trouw Nutrition) for the donations of fat supplements used.

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# The effect of two levels of nutrient intake on milk production of two dairy cow genotypes

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**Introduction** Breeding goals differ for different breeds of dairy cattle. For example the breeding programmes for Holstein-Friesian (HF) animals have been based primarily on improved milk production with little emphasis on functional traits such as fertility. In contrast, Norwegian (NC) dairy cattle have been bred using a multi-trait selection procedure for 25 years. These differences in selection procedures for the two breeds may have major effects on overall herd output and profitability with the forage-based systems of milk production employed in Northern Ireland. The objective of the present paper is to present the effects of two levels of nutrient input on food intake and utilisation, and animal performance of the two breeds, with diets based on grass silage during the first and second lactation.

**Materials and Methods** Thirty-two in-calf HF heifers ($PIN_{(00)}$ £44) and thirty-two in-calf NC heifers (total merit value = 10.1) were selected in Holland and Norway respectively and arrived at the Institute approximately 1 month prior to calving. These animals were representative of the top 1% and 5% of the HF cattle in the UK and NC cattle in Norway respectively. Post calving live weights for HF and NC cattle were 502 and 473; and 533 and 548 for years 1 and 2 respectively. Post calving, during year 1 the heifers were blocked into pairs within breed and allocated at random to either a low or high input system, based on grass silage for the two lactations. The low level of nutrient intake had forage:concentrate ratios of 70:30, 80:20 and 90:10, and 65:35, 75:25 and 85:15 for the first, second and third trimester of lactation 1 and 2 respectively. The high level of nutrient intake had forage:concentrate ratios of 40:60, 50:50 and 60:40 and 35:65, 45:55 and 55:45 for the first, second and third trimester of lactation 1 and 2 respectively. During year 2, 12 cows which had completed lactation 1, and 4 first lactation animals were used to evaluate the treatments. During year 1 the concentrates consisted of 230, 225, 300 and 245 g/kg fresh weight of barley, wheat, sugar beet pulp and soyabean respectively. During year 2 the concentrate consisted of 165, 165, 295, 195, 130 and 50 g/kg fresh weight of barley, wheat, sugar beet pulp, soyabean, maize gluten and rapeseed respectively. All cows received the equivalent of 160 and 180 g/day of a mineral/vitamin pre-mix during the first and second lactations respectively. The diets were offered as total mixed rations based on medium quality grass silage through Calan gates linked to a system of automatic cow identification and weigh cells.

**Results** The effects of level of feed input and cow genotype, reared in their country of origin, on food intake and animal performance are presented in Table 1. The HF genotype had significantly ($P<0.05$ or greater) higher food intake and yield of milk during both lactations and butterfat concentration ($P<0.001$) during the first lactation relative to the NC genotype. The NC genotype had significantly ($P<0.001$) higher condition score at the end of both studies. Increasing nutrient input increased ($P<0.001$) food intake, body condition score, milk yield and milk protein concentration during both lactations. Level of nutrient intake did not alter ($P>0.05$) milk fat concentration. There was a significant interaction between cow genotype and level of nutrient intake for the yield of milk during both lactations, fat plus protein during the first lactation and body condition score at week 20 in the second lactation. During both lactations, regardless of level of nutrient input, HF produced more milk energy output/kg DMI.

**Table 1** The effects of level of nutrient input and cow genotype on food intake and animal performance.

| | Level of nutrient intake (NI) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | High | | Low | | | Significance | | |
| Genotype | HF | NC | NF | NC | Sem | G | NI | GxNI |
| *Lactation 1* | | | | | | | | |
| TDMI (kg/d) | 16.6 | 15.1 | 12.9 | 12.2 | 0.28 | *** | *** | NS |
| Milk yield (kg 305 d) | 6840[c] | 5824[b] | 4992[a] | 4682[a] | 138 | *** | *** | *** |
| Fat (g/kg) | 45.0 | 42.5 | 44.1 | 40.5 | 0.84 | *** | NS | NS |
| Protein (g/kg) | 35.3 | 35.1 | 31.7 | 31.4 | 0.49 | NS | *** | NS |
| Milk energy (MJ/kg DMI) | 4.8 | 4.1 | 4.2 | 3.9 | | | | |
| Condition at d 305 | 2.57 | 3.11 | 2.22 | 2.67 | 0.062 | *** | *** | NS |
| *Lactation 2 (wk 1-20)* | | | | | | | | |
| TDMI (kg/d) | 18.9 | 17.5 | 14.9 | 14.2 | 0.52 | * | *** | NS |
| Milk yield (kg/d) | 35.8[c] | 28.9[b] | 26.0[a] | 24.4[a] | 1.17 | *** | *** | * |
| Fat (g/kg) | 40.9 | 42.0 | 42.9 | 39.9 | 1.16 | NS | NS | NS |
| Protein (g/kg) | 33.4 | 33.8 | 31.7 | 31.6 | 0.54 | NS | *** | NS |
| Milk energy (MJ/kg DMI) | 6.1 | 5.4 | 5.7 | 5.4 | | | | |
| Condition at wk 20 | 2.38 | 3.38 | 2.30 | 2.80 | 0.079 | *** | *** | ** |

**Conclusions** The HF cows produced greater quantities of milk and utilised nutrients more efficiently, but at the expense of greater tissue mobilization for milk production relative to the NC cows, the difference between the two genotypes being greater as the level of nutrient input increased.

# The effect of forage grinding and cutting height of urea treated whole crop wheat on the milk production and diet digestibility in dairy cows

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**Introduction** It has previously been demonstrated that feeding urea treated whole crop wheat to dairy cows results in a significant increase in dry matter intake but has little effect on milk yield (Sutton *et al*, 1997). Part of the reason behind this lack of response has been attributed to a decrease in digestibility, particularly that of starch. A forage mill has recently been developed which allow the grains to be ground prior to ensiling and potentially increase their digestibility. An alternative way to increase the energy value of whole crop wheat is to increase cutting/stubble height. Work by Weller *et al*, (1995) demonstrated an increase in calculated ME from 10.6 to 11.2 MJ/kg DM by increasing stubble height from 10cm to 40cm. The objective of the current experiment was therefore to determine the effects of forage processing (grinding) and cutting height at harvest of urea-treated whole crop wheat on the intake, milk production and diet digestibility in dairy cows.

**Materials and methods** A conventionally managed crop of winter wheat (*cv.* Equinox) was cut at two stubble heights (18 cm – long straw or 37cm – short straw) and the material either ground or unground at harvest to give four treatments: LU (long straw, unground), LG (long straw, ground), SU (short straw, unground) and SG (short straw, ground). Forty four Holstein-Friesian dairy cows (8 primaparous and 36 multiparous), on average 62 days into lactation were allocated to one of the four treatments. Whole crop wheat was mixed with 1$^{st}$ cut grass silage in the ratio 2:1 (DM basis). All cows were supplemented with 8.5 kg/d dairy concentrates (DM 871g/kg, ME 13.4 MJ/Kg DM and crude protein 200 g/kg DM) and 2kg/d rapeseed meal. Milk yields were recorded daily and composition weekly. Animals were weighed and condition scored weekly and blood sampled upon entering the trial and at weeks 12, 17 and 22 of lactation. Apparent digestibility was measured during week 21 of lactation for 5 cows per treatment using chromic oxide as an indigestible marker. Results were analysed as a 2x2 factorial design.

**Results** Increasing the cutting height at harvest (treatments SU and SG *vs.* LU and LG) increased the starch concentration of the forage from approximately 355 to 420g/kg DM whilst decreasing the fibre concentration from 420 to 340g/kg DM. Grinding significantly reduced ($P<0.05$) forage intake. Average forage DMI for animals fed ground forages (LG and SG) was 12.50 kg DM/d compared to 13.70 kg DM/d for animals fed unground forages (LU and SU). Shorter straw length also tended to decrease intakes but not significantly. Milk yield was not affected by grinding or cutting height. Butterfat concentrations were significantly reduced by decreasing straw length (average of 37.0g/kg for animals receiving short straw forgaes *vs* 41.8 g/kg for animals receiving long straw forages; $P<0.05$). Condition score was significantly higher in animals receiving short straw forages (average 2.8 for animals fed treatments SU and SG compared to 2.6 for animals fed treatments LU and LG; $P<0.05$). Grinding significantly ($P<0.001$) increased starch digestibility. Ground forage treatments had an average starch digestibility of 0.96 kg/kg compared to unground treatments with an average starch digestibility of 0.88 kg/kg. Blood glucose levels were significantly higher for animals fed short straw compared to long straw forages (mean value of 3.52 mmol/l for animals fed treatments SU and SG compared to 3.34 mmol/l for animals fed LU and LG; $P<0.05$) whilst ß-hydroxybutyrate (BHB) levels were lower for animals fed the short straw treatments ($P<0.01$).

**Table 1** Effect of cutting height and forage grinding on intake, milk production and apparent digestibility in dairy cows

| | | | | | | Significance | | |
|---|---|---|---|---|---|---|---|---|
| | LU | LG | SU | SG | s.e.d | Grinding | Height | Int |
| Forage intake (kg DM/d) | 14.09 | 12.97 | 13.32 | 12.03 | 0.834 | * | ns | ns |
| Milk yield (kg/d) | 30.8 | 29.9 | 30.0 | 29.8 | 0.82 | ns | ns | ns |
| Butterfat (g/kg) | 41.8 | 41.9 | 38.4 | 35.6 | 2.50 | ns | * | ns |
| Protein (g/kg) | 34.4 | 34.6 | 33.3 | 33.0 | 1.11 | ns | ns | ns |
| Liveweight (kg) | 587 | 616 | 607 | 619 | 21.8 | ns | ns | ns |
| Condition score | 2.59 | 2.61 | 2.83 | 2.76 | 0.108 | ns | * | ns |
| Digestibility (kg/kg) | | | | | | | | |
| NDF | 0.592 | 0.556 | 0.537 | 0.532 | 0.0458 | ns | ns | ns |
| Starch | 0.905 | 0.967 | 0.856 | 0.962 | 0.0174 | *** | * | ns |
| Glucose (mmol/l) | 3.36 | 3.32 | 3.50 | 3.53 | 0.093 | ns | * | ns |
| BHB (mmol/l) | 0.90 | 0.97 | 0.82 | 0.67 | 0.083 | ns | ** | ns |

**Conclusions** Grinding whole crop wheat at harvest significantly increased starch digestion. Cows responded by reducing intake. No significant effect was found in milk yield but milk fat concentrations (g/kg) decreased with reduced straw length.

**Acknowledgements** We are grateful to the Milk Development Council and MGA for funding this project.

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# Influence of Genetic Merit on 305-day Milk Production of Dairy Cattle on Commercial Farms at Three Levels of Feeding

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**Introduction** The objective was to estimate the influence of genetic merit on milk production at different levels of concentrate feeding. The presence of an interaction between genetic merit and level of feeding would mean that cows with a high potential for milk production are unable to express their potential under all conditions. Oldham and Simm, (1998) showed there was a significant interaction between level of concentrate feeding and $PTA_{f+p}$ under experimental conditions, and that the milk yield response to genetic merit increased with increased concentrate feeding. The current work estimated the value of genetic merit (PIN95 and $PTA_{f+p}$) on 305-day milk production traits of dairy cattle on commercial farms, where farms were categorised by the level of concentrate (Cg) fed.

**Materials and Methods** Model 1 was used to estimate the influence of PIN95, and $PTA_{f+p}$ on 305-day milk production. Two data sets of 1010 and 622 305-day records from dairy cattle monitored over a 2 1/2 year period between August 1997 and January 2000 were analysed to examine the influence of PIN95 and PTA scores on 305-day production traits, respectively.

Model 1 $Y = YOC + SOC + P + G + Cg + G*Cg$

Where Y is the dependent variable, milk, fat or protein yield; YOC is year of calving, SOC is season of calving, P is parity, G is genetic merit as defined by either PIN95 or $PTA_{f+p}$, Cg is level of concentrate feeding and * indicates an interaction. Level of concentrate feeding was defined as low (<7kg/d), medium (7-9kg/d) and high (>9kg/d). Medium and high Cg were made up of herds being fed TMR, while the low Cg was fed concentrate separately.

**Results** The mean response to genetic merit was 52.8kg milk/$PTA_{f+p}$ and 22.8kg milk/PIN95 (Table 1). The mean milk yields were 6811, 8821 and 8293 kg milk for low, medium and high input herds respectively. Table 1 shows the significant interaction between PIN95 and level of concentrate feeding for 305-day milk, fat and protein yields. The interaction between $PTA_{f+p}$ and level of concentrate feeding was not significant, however this analysis was carried out on a reduced data set (n = 622). An analysis using $PTA_{milk}$, $PTA_f$ and $PTA_p$ was also carried out and showed that the interaction $PTA_{milk}$*Cg was significant for milk and fat yields but not for protein yield and that the interaction $PTA_p$*Cg was significant for fat yield but not for either milk or protein yields. The results indicate a decrease in response to PIN95 at the high level of concentrate feeding, however the average ME densities of the diets for the three concentrate groups may explain this (11.0, 11.7 and 11.4 MJ ME/kg DM for low, medium and high concentrate groups respectively). The low ME density of the high concentrate group was due to forages being of lower quality. The results showed that for milk and protein yields there was no significant difference in response to PIN95 for low and medium groups. The L group was fed concentrate separately from forage and farmers may have fed more concentrate to high merit and less to low merit cows, and exaggerated the apparent response to PTA and PIN.

**Table 1: Estimates of influence of genetic merit on 305-day milk yield at three levels of concentrate feeding on commercial farms**

| Level of feeding | | | | Mean | Yield / $PTA_{f+p}$ | | Yield / PIN95 | |
|---|---|---|---|---|---|---|---|---|
| Cg | C kg/d | MEd | kg | Yields | b | se | b | se |
| Low | <7 | 11.0 | MY | 6811 | 57.9ab | 5.61 | 27.5a | 3.10 |
| Medium | 7-9 | 11.7 | | 8821 | 62.7b | 4.90 | 27.8a | 3.04 |
| High | >9 | 11.4 | | 8293 | 37.7a | 10.57 | 13.1b | 3.48 |
| Mean | | | | | 52.8 | 4.37 | 22.8 | 1.96 |
| *Significance of G*Cg* | | | | | *0.0980* | | *0.0013* | |
| Low | <7 | 11.0 | FY | 270 | 2.9 | 0.23 | 1.26a | 0.131 |
| Medium | 7-9 | 11.7 | | 350 | 2.4 | 0.20 | 0.76b | 0.128 |
| High | >9 | 11.4 | | 344 | 2.2 | 0.43 | 0.38c | 0.147 |
| Mean | | | | | 2.49 | 0.18 | 0.80 | 0.083 |
| *Significance of G*Cg* | | | | | *0.0980* | | *0.0001* | |
| Low | <7 | 11.0 | PY | 221 | 2.1 | 0.17 | 1.00a | 0.094 |
| Medium | 7-9 | 11.7 | | 291 | 2.2 | 0.15 | 1.05a | 0.092 |
| High | >9 | 11.4 | | 280 | 1.6 | 0.32 | 0.54b | 0.105 |
| Mean | | | | | 1.9 | 0.13 | 0.86 | 0.059 |
| *Significance of G*Cg* | | | | | *0.1605* | | *0.0003* | |
| n | | | | | 622 | | 1010 | |

Values with different letters are significantly different. Cg is the concentrate feeding group, C is the kg/d concentrate offered and G*Cg is the interaction between genetic merit and level of concentrate feeding, MEd is the ME density (MJ/kg DM) of the total diet offered, MY is milk yield, FY is fat yield and PY is protein yield. b is the regression coefficient

**Conclusions** Under commercial conditions milk production increased by 22.8kg milk per unit of PIN95 or 52.8kg milk per unit $PTA_{f+p}$, this was similar to the response in milk yield per unit $PTA_{f+p}$ (0.165kg milk/d or 50.3kg milk 305-days per $PTA_{f+p}$) found by Oldham and Simms (1998). The increase in production per unit of PIN95 was dependent on the level of feeding with the response per unit genetic merit increasing as level of feeding increased. Results highlighted the need for research to estimate the influence of genetic merit under different feeding systems and at constant levels of concentrate feeding.

**Acknowledgements** The authors wish to thank the South of England Agricultural Society and Wye College, University of London for financial support, and Philip Drury for help with the collection of data.

**Reference** Oldham and Simm, (1998), The Langhill Dairy Research Centre. *Journal of the Royal Agricultural Society of England* **159**: 94-106.

# Dairy cow performance in relation to the combination of cow height and condition score

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**Introduction** The increased use of Holstein genes in the British dairy herd led to the replacement of Friesian cows with the taller, thinner Holstein type. The objective of this study was to use measurements of cow height (Ht) at withers and average body condition score throughout lactation (ACS), to investigate their relationship with commercial herd performance.

**Materials and methods** During a 2½ year monitoring programme detailed records of production, health and fertility traits were collected from 1,424 Holstein-Friesian cows in 10 Kentish commercial herds. Milk production, somatic cell counts, dates of services and pregnancy diagnosis data were obtained from monthly milk recording data sheets. During monthly visits locomotion scores and body condition scores of individual cows were assessed. Height at withers was recorded once per lactation. Silage samples were taken and analysed, and used, along with the level of concentrate feeding, to estimate the energy density of the total diet. The model devised by Wicks and Leaver (2000) was used to estimate total daily dry matter intake per cow. Genetic information, in the form of index values and predicted transmitting abilities, was obtained from NMR records for each cow. Cows were allocated to three height (Htg; <140, 140-145 and >145 cm) and three average condition score groups (ACSg; <2, 2-2.5 and >2,5). Least-squares methodology was used to fit Model 1 to the data. A stepdown procedure was used in order to arrive at the final model appropriate for each trait, containing only the significant effects. Least-squares means were computed from the final model for all traits including Htg, ACSg and their interaction.

Model 1: $Y = H + Yr + MOC + L + Htg + ACSg + Htg* ACSg$

where Y was the dependent variable under analysis. The dependent variables analysed were milk yield, fat and protein yield (MY, FY, PY) and percent (F%, P%), dry matter intake (DMI), efficiency (Eff; ratio of ME for Milk to ME for maintenance, pregnancy and weight gain), days open (DO), number of services per conception (S), somatic cell count (SCC), locomotion score (LS), PIN and PTA for fat, protein and fat plus protein. In the models, H was the effect of the herd, YOC was the year of calving, MOC was the month of calving, L was the lactation number.

**Results** Table 1 shows that only five traits were affected by height but 12 were affected by average condition score. Least-squares means for the tall/thin cows are compared to those for small/fat cows in Table 1. Tall thin cows had better performance in the yield, efficiency and genetic traits. Small/fat cows had better protein percent, locomotion and a lower dry-matter intake. Height and condition score did not affect fertility traits.

**Table 1.** Trait means, significance of height and average condition score effects and least-squares means for the tall/thin and short/fat type cattle.

| Trait | Mean | Htg | ACSg | Tall/thin-type | SEM | Short/fat-type | SEM | P | Difference |
|---|---|---|---|---|---|---|---|---|---|
| MY (kg) | 8059 | NS | *** | 8957 | 168.8 | 7781 | 187.8 | *** | 1176 |
| FY (kg) | 324 | ** | *** | 353 | 7.2 | 310 | 8.0 | *** | 43 |
| PY (kg) | 265 | * | *** | 286 | 5.3 | 254 | 5.9 | *** | 32 |
| F% | 4.04 | NS | NS | 3.97 | 0.061 | 4.01 | 0.067 | NS | -0.04 |
| P% | 3.3 | NS | *** | 3.22 | 0.026 | 3.28 | 0.029 | *** | -0.06 |
| DMI (kg/d) | 18.0 | *** | *** | 18.6 | 0.26 | 16.8 | 0.31 | *** | 1.8 |
| Eff | 1.79 | *** | *** | 1.86 | 0.0413 | 1.72 | 0.0453 | *** | 0.14 |
| DO (d) | 111 | NS | NS | 116 | 8.5 | 119 | 9.4 | NS | -3 |
| S | 1.66 | NS | NS | 1.72 | 0.151 | 1.71 | 0.163 | NS | 0.01 |
| SCC (*000) | 142 | NS | NS | 203 | 24.5 | 197 | 27.2 | NS | 6 |
| LS | 1.51 | NS | * | 1.71 | 0.033 | 1.62 | 0.041 | * | 0.09 |
| PIN | 38.5 | ** | *** | 42.3 | 2.03 | 26.3 | 2.44 | *** | 16.0 |
| $PTA_{milk}$ | 318 | NS | *** | 367 | 21.2 | 218 | 21.8 | *** | 149 |
| $PTA_f$ | 10.2 | NS | *** | 12.7 | 0.79 | 6.89 | 0.839 | *** | 5.81 |
| $PTA_p$ | 9.65 | NS | *** | 10.9 | 0.62 | 6.489 | 0.639 | *** | 4.42 |
| $PTA_{f+p}$ | 19.8 | NS | *** | 27.7 | 1.83 | 14.0 | 1.82 | *** | 13.7 |

**Conclusions** The condition of cows had a greater effect on performance than height. The improved milk production brought about by the use of Holstein genes has probably been due their tendency towards lower condition and to improved efficiency rather than size.

**Acknowledgements** This study was funded by the South-East Agricultural Society and the Milk Development Council. The help of members of the Canterbury Grassland Study Group is gratefully acknowledged.

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# The effect of maturity of maize silage at harvest on the performance of lactating dairy cows offered two contrasting grass silages

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**Introduction** Under Northern Ireland conditions, until recently, it was difficult to achieve high starch maize (greater than 200 g/kg DM) more often than one year in fifteen. However, recent developments in maize breeding and in agronomic practices, particularly the development of degradable full cover plastic mulch, have resulted in the possibility of consistently producing high starch maize. As there is considerable variability in the quality of grass silage produced on Northern Ireland dairy farms, the objective of the present study was to examine the effects of maturity of maize silage at harvest on the performance of lactating dairy cattle offered two contrasting feed value grass silages. The potential concentrate sparing effect of contrasting maize silages was also examined.

**Materials and Methods** Four maize silages were offered in addition to two grass silages at a ratio of 40:60 maize:grass silage and supplemented with 7 kg concentrates/cow/day. The two grass silages were also offered as the sole forage and supplemented with either 7 or 11 kg concentrates/cow/day. The 12 treatments were offered to 24 lactating dairy cattle in a partially balanced, changeover design study. The maize silages were produced from crops planted on either 17 April or 20 May, either with or without complete plastic mulch cover and ensiled on 26 October, untreated. The high feed value grass silage was ensiled on 9 May, untreated after a 32 hour wilting period. The medium feed value silage was ensiled on 9 June after a 12 hour wilting period, treated with a bacterial inoculant. The forages were offered as total mixed rations through Calan gates linked to a system of automatic cow identification and weigh cells. The concentrate consisted of 75, 75, 140, 210, 300, 145, 30 and 25 g/kg of barley, wheat, maize gluten, sugar beet pulp, soya, rapeseed meal, molasses and minerals respectively.

**Results** The chemical composition of the maize and grass silages offered in the present study are presented in Table 1. There was a wide range in the dry matter (DM) and starch concentrations of the maize silages. The feeding value of the two grass silages differed considerably. Increasing grass silage feed value and concentrate feed level increased ($P<0.001$) total DM intake, milk and fat plus protein yields, and protein concentration. Grass silage feed value or concentrate feed level had no effect ($P>0.05$) on milk fat concentration. The effect of maturity of maize at harvest on animal performance is presented in Table 2. Regardless of maize silage DM concentration, inclusion of maize silage in the diet increased ($P<0.05$ or greater) forage and total DM intake, and fat plus protein yield. The inclusion of maize silage improved milk protein concentration, the effects of maize silage DM of 201, 280 and 384 being significant ($P<0.05$ or greater). Inclusion of maize silage improved milk yield, the response to maize silages with DM of 280 ($P<0.05$) and 384 ($P=0.06$) being significant. There was a significant negative relationship ($P<0.01$) between maize silage DM content and milk fat concentration. There was a significant interaction ($P<0.05$) between grass silage feed value and maize silage DM content for milk yield. Inclusion of maize silage DM of 201, 298, 280 and 384 altered milk yield by 1.0, 1.0, 1.6 and 0.7 kg relative to the medium feed value grass silage and by –0.2, 0.5, 0.7 and 1.31 for the high feed value grass silage. The potential concentrate sparing effect, as determined for milk yield was calculated to be 0.4, 0.9, 1.15 and 1.35 kg concentrate/cow/day for maize silage DM of 201, 298, 280 and 384 g/kg respectively.

**Table 1** Chemical composition of the maize and grass silages

| | Maize silage | | | | Grass silage feed value | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | Low | High |
| Dry matter (g/kg) | 201 | 298 | 280 | 384 | 193 | 304 |
| pH | 3.7 | 3.9 | 3.8 | 3.9 | 4.2 | 3.5 |
| *Composition of DM (g/kg)* | | | | | | |
| Crude protein | 107 | 74 | 77 | 69 | 142 | 182 |
| Ammonia (g/kg N) | 20 | 13 | 19 | 15 | 72 | 38 |
| Starch | 90 | 273 | 273 | 338 | - | - |
| Predicted D-value (g/kg DM) | - | - | - | - | 670 | 760 |

**Table 2** Effect of maturity of maize at harvest on food intake and animal performance

| | Grass silage | Maize silage DM (MS DM) (g/kg) | | | | Average | Significance | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | MS DM | | | | MS DM | GS x |
| | (GS) | 201 | 298 | 280 | 384 | sem | 201 | 298 | 280 | 384 | Linear | MS |
| Tot DMI (kg/d) | 16.9 | 18.2 | 18.1 | 18.5 | 18.4 | 0.44 | ** | ** | *** | *** | NS | NS |
| Milk (kg/d) | 26.8 | 27.2 | 27.5 | 28.0 | 27.8 | 0.54 | NS | NS | * | P=0.06 | NS | * |
| Fat (g/kg) | 39.8 | 42.4 | 41.4 | 41.7 | 40.2 | 0.88 | ** | P=0.09 | * | NS | ** | NS |
| Protein (g/kg) | 31.5 | 32.3 | 32.1 | 32.4 | 32.5 | 0.33 | * | NS | * | ** | NS | NS |
| Fat + protein (kg/d) | 1.89 | 2.00 | 2.08 | 2.02 | 2.02 | 0.046 | ** | ** | *** | * | NS | NS |

**Conclusions** Regardless of maturity at harvest, maize silage increased food intake and fat plus protein yield. Furthermore for high feed value grass silage, increasing maturity at harvest increased milk yield. Feeding maize silage has a concentrate sparing effect of up to 1.9 kg/cow/day depending on the feed value of the maize and grass silages.

# Relationship between linear and composite type traits, somatic cell count and lifespan in pedigree and non-pedigree cows

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**Introduction** Lifespan (LS) evaluations in the United Kingdom (UK) are based on bivariate analyses of LS scores, a direct longevity measure calculated from the number of lactations a cow has completed or is expected to complete, and an index of 4 linear type traits as an indirect predictor (Brotherstone et al, 1998). However, the study did not consider composite type traits or somatic cell count (SCC) and the data was only from pedigree herds. This study examines the relationship between type traits and SCC with LS in pedigree and non-pedigree cows.

**Materials and methods** LS scores were calculated from lactation data for cows born between 1986 and 1996 which were daughters of bulls with at least 87.5% Holstein genes. Cow records were matched with their sires' standardised breeding values for linear and composite type traits and Predicted Transmitting Abilities for SCC. The linear and composite conformation traits are as defined in the official Breed Society classification schemes in the UK (Animal Data Centre, 2001). The LS scores of daughters of bulls with at least 85% reliability for type and 50% reliability for SCC were extracted, together with their sires' proofs for type and SCC. The data set was divided into pedigree (PED), consisting of 595,922 pedigree daughter LS scores from 546 sires; and non pedigree (NPED) with 396,567 non-pedigree daughter LS scores from 448 sires. The relationship between type, SCC and LS was examined in each data set by regressing the daughter LS scores on sire proofs for type and for SCC; after correcting LS scores for herd-year-season, month and age at calving, and milk yield in first parity. These fixed effects were fitted in the regression model and multivariate analyses carried out. The regression coefficients from the analysis were used to calculate genetic correlations ($r_g$) between the type traits, SCC and LS, assuming a genetic standard deviation of 0.579 (Brotherstone et al, 1998) for LS.

**Results** The mean LS scores for PED and NPED cows were similar at 3.85 and 3.90 respectively. The $r_g$ between individual linear traits and longevity ranged from -0.27 for Body Depth to +0.31 for Udder Depth. Correlations were generally similar for PED and NPED cows. The most significant $r_g$ being Body Depth –0.18, Chest Width –0.20, Rump Width –0.21, Rear Legs Side –0.21, Udder Height 0.19 and Udder Depth 0.31. Traits which showed a different $r_g$ included Angularity (0.10, -0.07) in particular, Udder Support (0.18, 0.04) and Teat Placement (-0.07, -0.19), where the PED and NPED $r_g$ respectively are shown in brackets. Table 1 shows the regression coefficients between daughter LS scores and sire proofs for the composite type traits and SCC, plus the calculated genetic correlations.

**Table 1** Regression coefficients (RC) between daughter LS scores and sire proofs for composite traits and somatic cell counts, and the estimated genetic correlations

| | Pedigree data | | Non-pedigree data | |
|---|---|---|---|---|
| Traits | RC+ | Genetic correlations | RC+ | Genetic correlations |
| Legs and Feet | 0.059 | 0.22 | 0.048 | 0.18 |
| Body | -0.025 | -0.10 | -0.029 | -0.11 |
| Mammary System | 0.036 | 0.13 | 0.023 | 0.09 |
| Dairy | 0.015 | 0.06 | -0.022 | -0.09 |
| Total Merit | 0.092 | 0.28 | 0.018 | 0.07 |
| Somatic Cell Count | -0.007 | -0.23 | -0.006 | -0.19 |

+ All regression coefficients are significant ($P < 0.05$)

The regression coefficients for Total Merit and Dairy, and their $r_g$ with LS, were different for PED and NPED cows. The $r_g$ with LS were consistent across PED and NPED cows for Legs and Feet, Body, Mammary and SCC. The negative $r_g$ between Body and LS, together with the inconsistent $r_g$ across PED and NPED for Dairy, leaves Legs and Feet and Mammary as the most useful traits for indirect improvement of LS within an index. The consistent negative $r_g$ of SCC with LS in PED and NPED cows implies that higher SCC would result in a lower herd-life.

**Conclusion** The relationship between most linear traits and LS was similar for PED and NPED cows but different for Udder Support and Teat Placement Side. Feet and Legs and Mammary are the only two composite traits with consistent positive regressions coefficients in both PED and NPED cows and should be used as indirect predictors of LS within an index. While SCC should be included in an index in its own right, the study confirms that selection for lower SCCs will improve Lifespan.

**References**

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# Comparing the performance of Holstein and Friesian dairy cows on British dairy farms

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**Introduction** In recent years there has been an influx of Holstein genes into the UK dairy herd, largely achieved by a 'grading up' process using imported Holstein semen on Friesian cows. The research reported here investigates this process using performance records from UK dairy herds.

**Materials and methods** Measures of performance were derived from an analysis of 11 Friesian and 10 randomly selected farms using data supplied by Holstein UK and Ireland (HUKI). Over 12,000 cows and 45,000 lactation records, were collected between 1970 and 2001. Animals were characterised by the proportion (to the nearest 10%) of their genes derived from the two breeds. All farms contained purebred Friesian animals but the randomly selected farms displayed varying levels of Holsteinisation over the years. The 17 traits shown in Table 1 were analysed by least-squares techniques. Models were fitted to all traits (except longevity, number of lactations and age at first calving) comprising the effect of farm, year/month of calving, lactation number and %Holstein. Least-squares mean values for %Holstein were used to characterise the breeds and investigate trends in performance. Pure Friesian and Holstein cows were characterised as having 0 and 100% Holstein genes, respectively. A linear regression was fitted to each purebred annual least-squares mean in order to derive time trends in performance.

**Results** The results in Table 1 show that Holsteins had higher milk, fat and protein yields, associated PTA and index values and a lower age at first calving than Friesians. However, Friesians had higher milk composition values, and composition PTA, greater longevity and lower calving intervals than Holsteins. Figure 1 shows the mean milk yield of cows with different percentages of Holstein genes. There is evidence of negative heterosis for milk yield between these two breeds. The milk yield values of cows with between 20 and 80% Holstein genes appear to be linearly related and increasing at a similar rate to the line joining the two purebred values. The milk yield of purebred Friesians increased by 1,800kg between 1970 and 2000, about 57±3.02 kg/yr. Holstein milk yield increased by 128±21.8 kg/yr between 1990 and 2000. The effect of %Holstein on calving interval, fat and protein yield shows the same trend as milk yield (Figure 1). Protein percent was linearly (negative) related to increasing Holstein % whilst fat percent was only affected by Holstein % above 80%. This implies no heterosis in protein percent but positive heterosis in fat percent.

**Table 1.** Least-squares mean performance for pure Friesian and Holstein cows.

| | Holstein | sem | Friesian | sem |
|---|---|---|---|---|
| Milk yld (MY;kg) | 8069 | 59.9 | 6507 | 45.8 |
| Fat yld (FY; kg) | 296 | 2.43 | 249 | 1.86 |
| Prot. yld (PY; kg) | 249 | 1.89 | 211 | 1.45 |
| Fat % | 3.67 | 0.0185 | 3.85 | 0.0141 |
| Protein % | 3.09 | 0.00944 | 3.25 | 0.00723 |
| Calving Int (d) | 407 | 4.16 | 377 | 3.12 |
| MY PTA (kg) | 132 | 8.81 | -422 | 4.33 |
| FY PTA (kg) | 2.7 | 0.331 | -15.2 | 0.163 |
| PY PTA (kg) | 3.1 | 0.259 | -13.1 | 0.127 |
| F% PTA | -0.0378 | 0.00421 | 0.0283 | 0.00207 |
| P% PTA | -0.0175 | 0.00225 | 0.0132 | 0.00111 |
| PIN (£) | 6.8 | 0.701 | -34 | 0.344 |
| PLI (£) | 7.1 | 2.02 | -35 | 0.996 |
| CGI | 558 | 5.7 | 213 | 2.8 |
| Longevity (m) | 53.1 | 1.01 | 71.6 | 0.34 |
| No lactations | 2.37 | 0.012 | 3.65 | 0.0085 |
| Age 1st calv (m) | 28 | 0.20 | 31 | 0.08 |

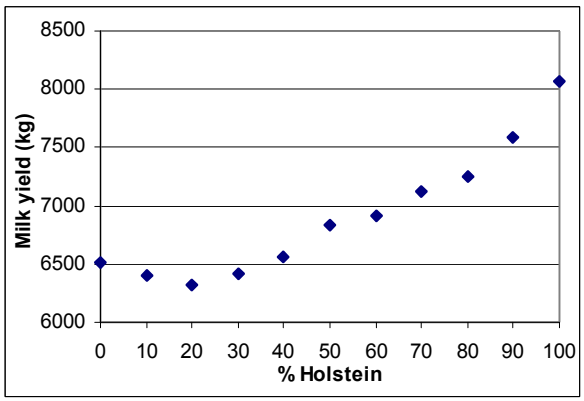


**Figure 1**. The effect of % Holstein on milk yield

**Conclusions** An analysis of on-farm performance of dairy cows has demonstrated clear differences between Holstein and Friesian cows. On average Holsteins have higher yield traits and associated PTA and index values, whilst Friesians have better fertility, longevity and milk composition traits. Both breeds have improved their performance in recent years. Crosses between Holsteins and Friesians demonstrate evidence of negative heterosis for yield traits but no heterosis for protein percent and positive heterosis for fat percent.

**Acknowledgements** This study was funded by the Milk Development Council. Data was supplied by Holstein UK and Ireland, whose help is gratefully acknowledged

# Application of test day model for the genetic evaluation of somatic cell counts and the effects of bimonthly sampling

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**Introduction** Currently, genetic evaluations in the United Kingdom for somatic cell count (SCC) are based on a single trait repeatability animal model (AM). A test day model (TDM), which allows for improved correction of environmental effects and the modelling of the individual lactation curve of each cow (Schaeffer and Dekkers, 1994), would be the preferred method of choice. In an attempt to reduce costs, farmers are opting for cheaper milk recording schemes, such as bimonthly recording. Bimonthly schemes might miss short frequency mastitis infection and it is not clear what effect this might have on evaluations for SCC. The objective of this paper is to report preliminary evaluations for SCC using a TDM and to evaluate the effect of bimonthly recording on such evaluations.

**Materials and methods** A data set consisting of 22,050,779 test day (TD) $\log_e$ SCC for the first three parities of 1,287,928 Holstein/Friesian cows was analysed with an animal random regression model. Herd-test-day and orthogonal polynomials of order 5 nested within age by season subclasses and within parity were fitted as fixed effects. Random animal and permanent environment effects consisted of orthogonal polynomials of order 2 and 3, respectively. Predicted Transmitting Abilities (PTAs) for SCC were calculated from the regression coefficients for animal effects and compared with those from the current AM system. A similar analysis was carried out for the Jersey breed based on 25,411 cows and 444,031 TD records (analysis A). For the Jersey breed, the analysis was repeated with the even numbered TDs of the daughters of 143 bulls deleted (analysis B) or odd numbered TDs deleted (analysis C). The results of analysis A were compared with analyses B and C to investigate the effect of using bimonthly TDs.

**Results** The correlations between the Holstein PTAs for SCC from the TDM and the AM were 0.91, 0.94, 0.95 and 0.97 for bulls with at least 20, 50, 80 and 100 daughters respectively. Similar correlations were obtained for the Jersey breed. This indicates the TDM will result in re-ranking of bulls, in particular for bulls with few daughters. Figure 1 shows the distribution of differences (AM-TDM) in PTAs for Holstein bulls with at least 50 daughters. The mean and standard deviation of the differences were 0.1 and 3.3 respectively. The latter confirms significant changes in the evaluations for some bulls. The TDM resulted in an increase in the accuracy of evaluations. Considering 919 Holstein bulls with at least 20 daughters; 94, 86 and 217 bulls had an increase of 12-18%, 10% and 6-8% in reliability respectively in the TDM relative to the AM.

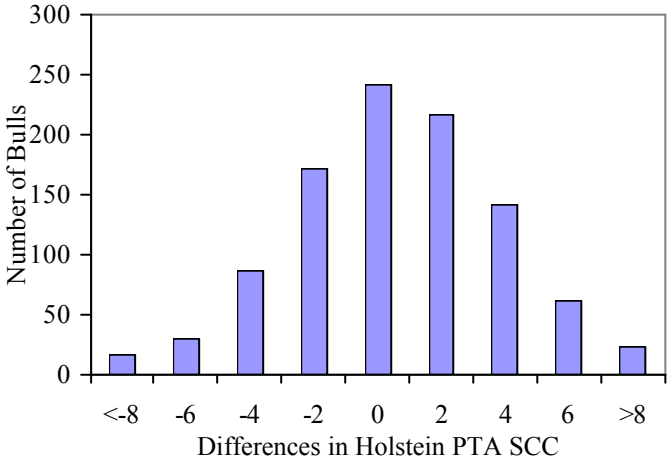


**Figure 1** Distribution of differences AM – TDM

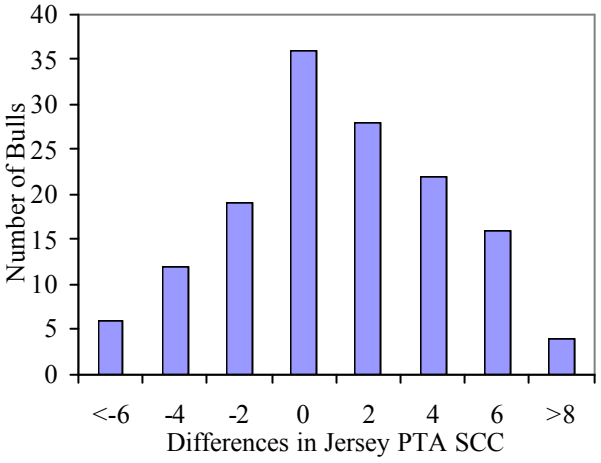


**Figure 2** Distribution of differences for bull SCC PTAs between analyses A and B

Figure 2 shows the distribution of differences (A-B) in Jersey bull PTAs between analyses A and B. The exclusion of even numbered TDs resulted in both over and under-prediction of bull PTAs but there was a greater tendency towards under-prediction. The correlation between SCC PTAs from analyses A and B for 143 Jersey bulls was 0.89, indicating considerable re-ranking as a result of using bimonthly TD. A similar correlation of 0.87 was found between A and C. These correlations increased to 0.93 if bulls with at least 150 daughters were considered. The use of bimonthly TD resulted in a mean reduction in reliability of 8% for the 143 bulls or 10% for 72 bulls with, at most, 50 daughters. The correlation of bull PTAs between analyses B with C was 0.99, indicating that exclusion of even or odd numbered TDs had similar effects.

**Conclusion** The introduction of TDM evaluations for SCC will result in re-ranking of bulls and a considerable gain in the accuracy of evaluations, especially for bulls with few daughters. The use of bimonthly information reduced reliability by up to 10% and introduced considerable re-ranking of bulls due to changes in PTAs.

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## Serum urea concentration to determine protein requirements of pig genotypes

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**Introduction** An *in vivo* procedure to determine nutrient requirements for growing pigs would have substantial cost advantages over serial slaughter and furthermore repeated measurements would allow estimation of protein requirements at each stage of growth. Serum urea concentration (*Urea*) has been proposed as a candidate indicator of protein requirement as over-supply of protein will increase *Urea* (Chen *et al*., 1995). The study estimated genetic variation in *Urea* at different stages of performance test and the relationships between *Urea* and performance traits.

**Material and methods** The study consisted of 230 Large White pigs from the divergent selection lines of the Edinburgh lean growth experiment (Cameron, 1994). There were 50 pigs in the lean growth (LGA), daily food intake (DFI) and lean food efficiency (LFC) selection groups performance tested on a phase feeding regime and 40 pigs in the LGA and DFI groups tested with a diet-choice procedure, with equal numbers of animals in the high and low selection lines. The study included isoenergetic diets (14.0 MJ DE/kg DM) differing in ileal lysine : energy (P, Q, R, S, T, U and X : 0.6, 0.7…1.2 g/MJ DE). The performance test with phase-feeding consisted of three 14-day periods, starting at 30, 50 and 75 kg, with pigs ad-libitum fed diets R, S, T, U or X, then Q, R, S, T or U and finally P, Q, R, S or T with diet S fed between time periods. Diets Q and U were available to diet-choice tested pigs. *Urea* was measured in phase-fed pigs at the start and end of each test phase and in diet-choice pigs at 30, 50 75 and 90 kg. Serum urea concentrations, expressed as mg/dl, were determined using a commercially available assay (Randox, Co. Antrim). Protein gain was predicted by 0.16 Δliveweight - 0.11 Δbackfat depth, where Δ is the change in trait during test phase, with the equation derived from a previous serial slaughter study. Data were analysed with residual maximum likelihood methodology with effects fitted for sex, diet, selection line and their interaction while laboratory assay and litter were fitted as random effects as litters consisted of 7 animals with 2 allocated to diet-choice and 5 phase fed on different diets.

**Results** Between selection line-diet subclass variances at the start (0, 3, 9) and end (29, 43, 104 $(ml/dl)^2$ ) of test phase indicated little variation in *Urea* prior to test phase with increased variation due to diet. There were no between-diet differences in *Urea* at the end of the first test phase, but pigs fed higher lysine : energy diets had higher *Urea* at the end of the third test (*Urea* for diets P Q R S T : 20, 27, 38, 40, 41; s.e.d. 2.0 mg/dl) (Figure 1). There was no stage of test effect on *Urea* for diet-choice pigs with an effective lysine :energy of 0.74 throughout performance test (Figure 1).

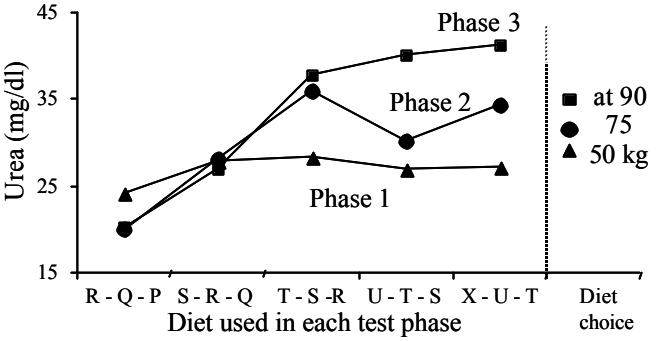


**Figure 1** *Urea* at the end of each test phase by diet

*Urea* had a heritability of 0.33, 0.30 and 0.28 (s.e. 0.19) at the end of each test phase. Correlations between *Urea* and lysine intake during each test phase were similar for phase-fed and diet choice pigs (0.34, 0.46 and 0.55, s.e. 0.10). Predicted protein gain was uncorrelated with *Urea* for phase-fed pigs (0.00, 0.07 and 0.06) and for diet-choice animals (-0.08, -0.13 and -0.02). *Urea* responses to dietary change at the end of test phase 3 were essentially parallel for the selection lines (Figure 2) and the quadratic terms were not significantly different from zero. Generally, selection lines with low genetic merit for lean growth (eg low LGA and LFC and high DFI) had higher *Urea* than the complementary selection lines. The high LGA line had significantly higher *Urea* than the low LGA line when diets R and S were fed (54 v 40, s.e.d. 6 mg/dl).

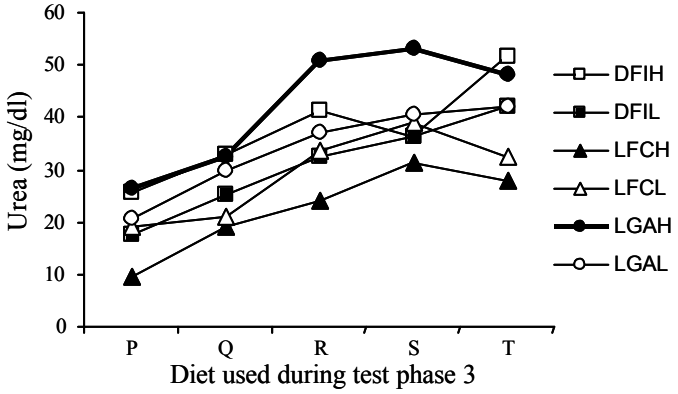


**Figure 2** *Urea* of selection lines at the end of test phase 3

**Conclusions** Selection line responses in *Urea* to dietary change were consistent with the concept of *Urea* as a predictor of protein deposition, but the high LGA line response suggested that use of *Urea* may be selection strategy specific, so imposing conditions on the conclusions of Chen *et al*. (1995). For example, high protein turnover as well as low protein requirement may result in high *Urea*. Although *Urea* was heritable and correlated with lysine intake it was uncorrelated with protein gain, indicating that *Urea* will be of limited use as a predictor of genetic merit for protein requirement.

**Acknowledgements** The research project was funded by the Department for Environment, Food and Rural Affairs.

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# A genetic analysis of wool and lamb production traits in Scottish Blackface Sheep

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**Introduction** Scottish Blackface sheep have a multi-purpose role in the UK to produce breeding females and lambs for meat consumption. Over the last fifty years, wool has accounted for a very low proportion of economical return from hill sheep production in the UK. In recent years, the ratio of the value of lamb meat to wool clip has altered, with wool becoming relatively more important in particular for hill breeds. The consequence of direct selection for improved carcass traits in these breeds on wool quality is unknown. With other sheep breeds such as Merino, selection for improved wool traits has largely been undertaken with little regard to the impact on meat production. The objectives of this study are to quantify wool quality traits and examine genetic relationships between wool quality and carcass traits in Scottish Blackface sheep.

**Materials and Methods** Data from a wider research programme to investigate the consequences of breeding for improved carcass and maternal characteristics (Conington *et al*., 1998) were used to assess the relationships between lamb growth, carcass measurements, and fleece characteristics, assessed at different ages. The data set consisted of 2,524 records on male and female lambs born between 1994 and 2000. Production traits recorded were liveweights at birth, (BWT), 'marking' (MWT) at an average age of 7 weeks, weaning (WWT) at an average age of 17 weeks, ultrasonic fat (AVFD) and muscle depths (MD) at the 3rd lumbar vertebra, taken at weaning time, slaughter weight, conformation score (CONF) and fat class (FATC), as assessed on the carcass after slaughter. Animals were slaughtered when they reached a target condition score of 3. Wool traits were assessed on live lambs by the same British Wool Marketing Board assessor each year and included staple length (SL) in cm, KEMP, BLACK and GREY fibre contamination and overall recommendation of the fleece (REC). The latter was a 3-point score designed to encompass all wool quality assessments. Kemp, grey and black were recorded using a 10-point score, and RECC was closely associated with these traits. Birthcoat length (BCT) was recorded in cm within 24 hours of birth, with all other wool traits assessed at an average of 20 weeks of age. Wool quality traits and fat class were transformed to account for skewed distribution of these measurements. Using multiple regression in GENSTAT, the fixed effects identified as important sources of variation included farm, genetic line, age of dam, sex, year of birth and birth and rearing rank. REML was then used to estimate the fixed effect means. Variance components were estimated using a sire model in ASREML (Gilmour *et al*., 1998), fitting the fixed effects listed above. Heritability and genetic correlations were predicted using univariate and bivariate analyses respectively.

**Results** Heritabilities for wool quality traits were high, with low standard errors, particularly for birthcoat length. The genetic correlations between kemp and lamb weights, muscle depth and conformation are high and well-estimated. RECC follows a similar pattern. Moderate genetic correlations exist between all wool quality traits and carcass conformation, i.e. better conformation animals have better wool quality. Lambs with longer birthcoats have less fat, deeper muscle depths and better conformation. Some antagonism exists between length of wool staple (measured at birth and at 20 weeks) and post-birth live weight measurements. Large standard errors limit the interpretation of some correlations.

**Table 1** Genetic correlations (standard errors) between liveweight, carcass traits and wool traits and heritablity ($h^2$) estimates for wool traits in lambs

| | BWT | MWT | WWT | AVFD | MD | SLWT | CONF† | FATC | $h^2$ |
|---|---|---|---|---|---|---|---|---|---|
| SL (cm) | 0.084 | -0.27 | -0.18 | 0.15 | -0.30 | -0.34 | 0.53 | -0.35 | 0.62 |
| | (0.14) | (0.13) | (0.14) | (0.15) | (0.12) | (0.13) | (0.11) | (0.14) | (0.11) |
| BCT(cm) | 0.12 | 0.014 | -0.04 | -0.27 | 0.302 | -0.07 | 0.32 | -0.288 | 0.69 |
| | (0.15) | (0.14) | (0.13) | (0.15) | (0.13) | (0.15) | (0.13) | (0.16) | (0.11) |
| KEMP‡ | 0.14 | 0.44 | 0.48 | -0.05 | 0.42 | 0.59 | -0.37 | -0.015 | 0.46 |
| | (0.15) | (0.12) | (0.13) | (0.16) | (0.12) | (0.11) | (0.13) | (0.16) | (0.09) |
| BLACK‡ | 0.016 | -0.51 | 0.36 | -0.55 | 0.03 | 0.39 | 0.41 | -0.15 | 0.26 |
| | (0.33) | (0.24) | (0.31) | (0.28) | (0.33) | (0.29) | (0.31) | (0.33) | (0.13) |
| GREY ‡ | -0.39 | 0.02 | 0.01 | -0.23 | 0.37 | 0.005 | -0.45 | 0.38 | 0.37 |
| | (0.13) | (0.15) | (0.16) | (0.16) | (0.13) | (0.15) | (0.12) | (0.17) | (0.08) |
| REC§ | -0.014 | 0.39 | 0.37 | -0.06 | 0.44 | 0.44 | -0.40 | -0.022 | 0.31 |
| | (0.16) | (0.14) | (0.14) | (0.17) | (0.12) | (0.13) | (0.13) | (0.17) | (0.07) |

†MLC Conformation score where 1=E,2=U,3=R,4=O,5=P; ‡ Scale 1-10 where higher scores indicate better quality (i.e. less kemp, grey or black wool); § Recommendation score where 0=not recommended, 1=recommended, 2=highly recommended.

**Conclusion** From these results, it can be seen that selection for improved carcass traits in hill breeds would not be detrimental to wool quality. Selection for heavier lambs at slaughter is expected to result in lambs with shorter staple lengths.

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**Acknowlegements:** MLC, SEERAD, BWMB and the Worshipful Company of Woolmen.

# QTL Detection in the UK Suffolk and Texel Sheep Sire Referencing Schemes

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**Introduction** Genomic research and the detection of quantitative trait loci (QTL) provide tools to enhance genetic progress and improve understanding of the biology of commercially important traits. The large sire reference schemes in UK terminal sire sheep breeds provide a unique opportunity to investigate QTL segregation within commercial populations. This study aims to identify QTL for performance traits in commercial Suffolk and Texel sheep.

**Material and Methods** Blood samples were collected from three sire reference scheme (SRS) Suffolk rams (S1-S3) and five SRS Texel rams (T1-T5) and a proportion of their commercial progeny. Progeny group size varied between 66-230 individuals. DNA was extracted from blood and sires were genotyped for up to 9 candidate regions on chromosomes 1, 2, 3, 4, 5, 6, 11, 18 and 20 based on current knowledge and other studies of the sheep genome. Markers heterozygous in each sire were genotyped in all their respective half-sib progeny. The probability of inheriting a particular sire chromosome at a particular position was calculated for each offspring at 1cM intervals, using the method of Knott *et al.* (1996). Phenotypic measurements of weight at 8 weeks of age (8Week) and ultrasonic scanning (ScanWT), ultrasonic muscle (Mus) and fat (Fat) depth at the third lumbar vertebra were obtained from Signet. Additionally, both muscle and fat depth were adjusted to correct for body weight (MusWT and FatWT). Phenotypes were corrected for fixed effects of flock-year, sex, birth-rearing rank and age of dam, estimated using ASREML (Gilmour *et al*., 1999). In addition, ultrasonic scanning traits were corrected for age at scanning. Each of the adjusted phenotypes was regressed on the inheritance probabilities, at each location along each chromosome for each family. For each regression an F-ratio of the model including the phase probability versus the same model without the phase probability was calculated. The best estimated position for a QTL in each family, for each trait, was taken to be the location with the largest F-ratio. Following the recommendations of Lander and Kruglyak (1995) for confirmed linkage, a threshold representing a nominal P value of 0.01 was used.

**Table 1** Summary of significant effects at the 1% nominal level. Effects given in kg for weight traits and mm for fat and muscle traits

| Chromosome | Sire | Trait | Position | Effect (se) | F |
|---|---|---|---|---|---|
| 1 | S1 | Mus | 200 | 2.28 (0.729) | 9.77 |
| | | MusWT | 200 | 1.62 (0.547) | 8.78 |
| 2 | T1 | Fat | 169 | 0.62 (0.170) | 12.85 |
| | | FatWT | 169 | 0.51 (0.160) | 9.94 |
| | T2 | MusWT | 59 | 1.24 (0.463) | 7.14 |
| | T4 | 8Week | 167 | 1.93 (0.617) | 9.78 |
| | | ScanWT | 165 | 2.74 (0.997) | 7.53 |
| 3 | S2 | FatWT | 80 | 0.44 (0.154) | 7.96 |
| | T3 | Mus | 13 | 1.54 (0.433) | 12.64 |
| | | MusWT | 18 | 1.25 (0.340) | 13.47 |
| 4 | T2 | FatWT | 43 | 0.75 (0.221) | 11.44 |
| 18 | S1 | 8Week | 98 | 2.59 (0.759) | 11.69 |
| | S2 | 8Week | 38 | 1.52 (0.572) | 7.05 |
| | | ScanWT | 44 | 2.10 (0.754) | 7.77 |
| | T2 | Mus | 85 | 1.82 (0.554) | 10.79 |
| | | MusWT | 88 | 1.26 (0.441) | 8.16 |
| | T5 | Fat | 75 | 0.77 (0.289) | 7.02 |
| | | FatWT | 76 | 0.69 (0.241) | 8.19 |
| 20 | T1 | Fat | 49 | 0.59 (0.182) | 10.44 |

**Results** Significant effects were detected in 6 different chromosomal regions. These are summarised in Table 1. Effects varied between 0.5-0.8 phenotypic standard deviations. Four of the regions contained effects only detected in individual sires. In contrast, the effects on chromosomes 2 and 18 were supported across 2 or 3 different sires. The data on chromosome 2 suggests 2 QTL (results not shown), one affecting muscle growth around 60cM and another affecting fat growth around 170cM. The fat QTL on chromosome 2 corresponds to the region of the myostatin locus responsible for double muscling in cattle. The effects on chromosome 18 on muscle in T2 are almost identical to the description of the Carwell gene, both in size of effect and chromosomal position, possibly suggesting the Carwell locus is segregating in UK Texel sheep.

**Conclusions** The study has been successful in detecting QTL in UK sire reference scheme sheep populations. The chromosomal candidate region approach is useful in populations where traditional QTL mapping methods e.g. diverse breed or line crosses, may not be feasible or appropriate. Future work aims to improve the resolution of position and effects detected in this study.

**Acknowledgements** The UK sheep genome mapping project is funded by the Department for Environment, Food and Rural Affairs (DEFRA), Scottish Executive Environment and Rural Affairs Department (SEERAD) and the Meat and Livestock Commission (MLC). Contributions are made by Elite Texel Sires (UK) Ltd., Suffolk Sire Referencing Scheme Ltd. and Charollais Sires. The authors acknowledge additional assistance from Signet and Edinburgh Genetics.

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# Supplementation of sow diets with plant extracts enhances piglet growth prior to weaning

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**Introduction** Sow lactation performance is critical to piglet survival and pre-weaning growth. Effective lactogenesis combined with strong piglet vigour at birth will help prevent hypoglycaemia and mortality. In addition, immune protection of the sucking pig is dependant on the acquisition of immunoglobulins from the colostrum of the sow. High milk output in late lactation will ensure greater weaning weights, since piglet growth potential exceeds milk supply by day 10 of lactation (Harrell *et al*, 1993). Maximising sow feed intake ensures maximum milk production. A variety of plant extracts are recognised for their health and growth promoting properties which include stimulation of the digestive and immune systems (Platel and Srinvansan, 1996). The objective of this study was to evaluate the ability of various plant extracts to stimulate piglet and sow performance when used as supplements in the diets of lactating sows. Three extracts were selected for their potential benefits to health, appetite and digestion, these being Yucca Shidigera, Quillaja Saponaria and Combination (a blend of the spices capsicum 1.0%, cinnamaldehyde 1.25 % and oregano oil 0.85 %).

**Materials and methods** Eighty JSR hybrid sows of mixed parity, liveweight and fatness were housed in conventional indoor farrowing crates from day 107 of gestation until weaning. Sows were allocated according to parity, liveweight, fatness and past reproductive performance, to one of four dietary treatments: 1. Control, 2. Combination (100g/tonne feed), 3. Yucca (200g/t), 4. Quillaja (250g/t). Diets were otherwise identical, formulated to contain 14.0 MJ DE/kg and 10.0g total lysine/kg. Prior to farrowing (days 107-114 of gestation) sows received 2.5kg feed/day of their respective diet. During lactation feed was offered *ad-libitum* with sow feed intake (FI) recorded daily. At farrowing piglets were individually weighed and ear tagged. Piglet liveweight was recorded on days 1, 7, 14 and 21 and at weaning which took place at 23±*2.5* days of age. Sow P2 backfat was measured weekly from parturition to weaning. Data were corrected for litter size and birth weight and analysed using the GLM procedure of Minitab 12.2.

**Results** Piglet growth rates between birth and day 1 and days 15-21 were significantly affected by sow treatment, with litters from sows receiving the Combination supplement having the greatest advantage *(P<0.01,* Table 1*)*. Piglet weights on day 21 of lactation were also greater for the Combination supplemented sows compared with all other treatments *(P<0.05)*. Sow FI was unaffected by treatment at any time during lactation as was sow P2 backfat loss between parturition and weaning.

**Table 1.** *Piglet average daily growth (ADG, g/pig/day), piglet weights on day 21 (g), sow average daily feed intakes (FI, kg/day) from parturition to day 21 of lactation and sow P2 backfat loss (mm) between farrowing and weaning.*

| Treatment | Piglet ADG (g/day) | | | | Sow Feed Intake (kg) | | | | Sow P2 |
|---|---|---|---|---|---|---|---|---|---|
| | Days 0-1 | Days 1-14 | Days 15-21 | Day 21 Wt (g) | Days 0-7 | Days 8-14 | Days 15-21 | Total | loss (mm) |
| Control | 99[b] | 233 | 246[b] | 6584[b] | 3.15 | 5.41 | 6.00 | 102.1 | 2.89 |
| Combination | 117[a] | 238 | 290[a] | 6878[a] | 3.53 | 5.84 | 5.61 | 103.9 | 3.49 |
| Quillaja | 77[c] | 237 | 235[c] | 6330[c] | 3.34 | 5.51 | 5.90 | 102.9 | 3.60 |
| Yucca | 107[b] | 231 | 255[b] | 6498[b] | 3.46 | 5.44 | 5.68 | 105.6 | 3.08 |
| SEM | 7.9 | 5.6 | 9.3 | 132 | 0.19 | 0.27 | 0.34 | 4.05 | 0.47 |
| Sig. | ** | ns | *** | * | ns | ns | ns | ns | ns |

Means in the same column without a common superscript differ significantly: **(P<0.05), **(P<0.01), ***(P<0.001).*

**Conclusions** Inclusion of the Combination extract in the sow diet during lactation enhanced piglet performance. The superior growth rate of these piglets in the first 24 hours of life suggests facilitation of lactogenesis or enhanced piglet vigour immediately after birth. Although there were no treatment differences over the next two weeks, during the third week of lactation, when sow milk yield normally becomes limiting to piglet growth, the Combination additive again led to improved piglet weight gains. This suggests that milk yield had been increased, however since sow FI and sow backfat loss remained unchanged, it may indicate greater efficiency in the utilisation of feed for milk production. Alternatively, or additionally, piglets may have been utilising the milk with greater efficiency resulting in increased growth.

**Acknowledgements** This work was funded by Axiss France S.A.S.

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# Removal of both zinc oxide and avilamycin from the post-weaning diet has a detrimental effect on pig performance through to slaughter

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**Introduction** Avilamycin (antibiotic growth promoter) and zinc oxide are both included in the diets of newly weaned piglets to enhance growth performance and reduce the incidence of diarrhoea (MLC, 2000). It is thought that both compounds positively influence the bacterial populations residing in the gastrointestinal tract. However, growing concerns regarding antibiotic resistance and environmental pollution are likely to result in the banning of these dietary additives within the EU. This experiment, therefore, aimed to investigate what effect removing both avilamycin and zinc oxide from the post-weaning diet would have on the growth performance of weaned piglets.

**Materials and methods** Fifty two crossbred piglets (JSR Healthbred) were weaned, at 21.2 ± 0.30 days of age (± SEM) and 6.9 ± 0.16 kg liveweight, into commercial, fully-slatted flatdeck accommodation. Six or 7 piglets were allocated to each pen (1.99$m^2$) on the basis of weight, litter and sex. Four pens were randomly allocated to one of two dietary treatments. Treatments were: 1) Control - no supplementation, and 2) ZnO + AGP - supplemented with 3100mg ZnO/kg feed and 40mg/kg avilamycin (Maxus™). Both diets were formulated to contain 17.5 MJ DE/kg, 17.5 g total lysine/kg from weaning to day 7, and 16.0 MJ DE/kg, 16.0 g total lysine/kg between days 8 and 20. Thereafter, all trial pigs received the same grower (14.0 MJ DE/kg, 13.5 g total lysine/kg) and finisher (13.0 MJ DE/kg, 11.5 g total lysine/kg) rations. All diets were provided *ad libitum.* From day 20 onwards, the trial pigs were housed in conventional grower (d21-60) and finisher (d61-118) accommodation. Piglets were tagged and individually weighed at weaning and days 20, 60 and 118 post-weaning. Daily feed intake per pen was recorded between weaning and day 20. Faecal samples were obtained from selected piglets at weaning and day 19. The samples were cultured on fresh blood agar in the presence and absence of $O_2$ to obtain a total bacterial count. Data were analysed using the GLM procedure of Minitab 12.2.

**Results** Pig performance is shown in Table 1. From days 1-20, the ZnO + AGP piglets consumed more feed/pig/day ($P<0.01$), gained more weight/pig/day ($P<0.001$) and had better FCR ($P<0.05$) than Control piglets. As a result, the ZnO + AGP pigs were heavier than Control pigs by day 20 post-weaning (13.0kg vs 11.7kg, $P<0.001$) and this weight advantage increased through to day 60 ($P<0.01$). ZnO + AGP pigs were numerically heavier than the Control pigs at slaughter (day 118, 82.4kg vs 79.3kg), but this difference was no longer significant. Total faecal bacterial counts were similar at weaning, however, by day 19 the ZnO + AGP pigs had a lower total faecal bacterial count than the Control pigs ($P<0.05$).

**Table 1** *Pig start weights, day 1-20 average daily feed intakes (FI, g/pig/day), average daily liveweight gains (ADG, g/pig/day), feed conversion ratio (FCR), day 19 total faecal bacterial counts and liveweights on days 60 and 118*

| | Start Wt (kg) | Day 1-20 FI | Day 1-20 ADG | Day 1-20 FCR | D19 faecal bacterial count (CFU/g faeces) | Day 60 Wt (kg) | Day 118 Wt (kg) |
|---|---|---|---|---|---|---|---|
| Control | 6.9 | 245 | 237 | 1.04 | $1.69x10^8$ | 39.0 | 79.3 |
| ZnO + AGP | 6.9 | 299 | 306 | 0.98 | $4.43x10^7$ | 42.5 | 82.4 |
| SEM | *0.16* | *4.79* | *2.61* | *0.01* | *$3.42x10^7$* | *0.71* | *2.69* |
| Significance | NS | ** | *** | * | * | ** | NS |

NS non-significant, *(*P<0.05*), **(*P<0.01*), *** (*P<0.001*)
CFU – colony forming units

**Conclusion** The omission of both zinc oxide and avilamycin from the post-weaning diet had a detrimental effect on post-weaning and subsequent growth performance. The bacteriological data demonstrated that the enhanced growth performance of the ZnO + AGP supplemented piglets in the initial 20 days post-weaning coincided with a reduction in their total faecal bacterial load in comparison to their non-supplemented counterparts. This suggests that enhanced growth may result from a reduction in nutrient competition between the host and the resident gut bacteria, a suggestion which is supported by the improvement in FCR observed for the ZnO + AGP supplemented group during this period (days 1-20). The benefit of ZnO + AGP inclusion extended beyond the initial supplementation period and in this trial would have equated to a reduced days to slaughter for a specified slaughter liveweight. The results of this experiment indicate the importance of antimicrobial additives in existing management systems and emphasise the need for effective alternatives should current growth promoting dietary additives be phased out.

**Acknowledgements** This work was funded by Primary Diets Ltd.

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# Predicting food intake and performance during adaptation to a new food.

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**Introduction** Current models that predict food intake over time assume that the animal is always fully adapted to the food that it is on. This approach does not account for the immediate effects of a change in food type upon intake. Such a change may have large effects on intake and performance particularly when the change is to a food of higher bulk content. Such a change initially causes a reduction in intake. Over time, with adaptation, intake gradually increases until a new equilibrium intake is reached. The ability to predict intake and performance during the period of adaptation will allow models to predict food intake on high bulk foods more accurately. Data recorded during the period of adaptation are frequently excluded, perhaps to make the prediction of intake on high bulk foods easier. This work is an attempt to develop a model to predict intake and performance during the adaptation period when an animal is changed to a food of higher bulk content.

**Materials and methods** The system being considered is a growing pig fed *ad libitum* on a single homogenous food balanced for nutrients with no toxins so that the first limiting resource is energy. The scale of energy used to estimate requirements is the effective energy scale (Emmans, 1994). It is assumed that an animal has a desired rate of intake (DFI) which is determined by the requirements for protein and lipid depositions and maintenance. DFI may not be achieved if a constraint to intake, such as the bulk content of the food, exists. Where a bulk constraint operates intake is calculated as the constrained rate of intake (CFI) where CFI = $C_{WHC}$/WHC g/day, where $C_{WHC}$ is the animals capacity for WHC (units/ kg liveweight day), and WHC (g water/g dry food) is the water holding capacity of the food. Actual intake is predicted to be the lower of DFI and CFI. Where intake is not constrained it is assumed that genetic potential will be achieved. Potential growth (g/day) is described by the Gompertz growth function. Where intake is constrained growth will be less than the potential. The actual LWG is predicted as $(dW/dt)_{con} = (EEI-EE_m)/e_g$ g/day, where EEI is the energy intake (MJ/day), $EE_m$ is the energy required for maintenance (MJ/day) and $e_g$ is the energy required for gain (MJ/kg). Actual growth is predicted to be the lesser of potential and constrained growth. To deal with adaptation we assume that the time taken to reach equilibrium depends on the difference in WHC between the first and second foods and that the capacity to consume food bulk is related to the WHC of the current food. It is proposed that the capacity for WHC on the first day of a new food will be equal to the current capacity for WHC on the last day of the previous food so that $C_{WHC}$ = (FI.WHC)/W g/kg. Thereafter $C_{WHC}$ will gradually increase over time to a maximum. The rate of change in $C_{WHC}$ is assumed to be the same for all pigs regardless of the food being fed. The increase in capacity over time is assumed to be linear at the rate of 0.01 units/day. The data used to evaluate the model were taken from Kyriazakis and Emmans (1995) and Whittemore et al. (2001) where young growing pigs were fed a series of foods differing in bulk content

**Results**. The model is able to reproduce the experimental data with a reasonable degree of accuracy (Figure 1 and 2). The model accurately predicts changes in intake when there is a change from a more to a less bulky food (Figure 2), but underpredicts intake on the less bulky food when there is a change to a food high in wheatbran content (Figure 1).

**Conclusions** The model was designed to predict changes in intake during the period of adaptation to a new food of higher bulk content. The assumptions made in the model about the way in which intake changes during the adaptation period are an initial attempt at describing this phenomenon. The predictions of the model do however, reflect the experimental data. The underlying theoretical assumptions of the model are assumed to be reasonable. The model shows that progress on the control of intake bulky foods has been made.

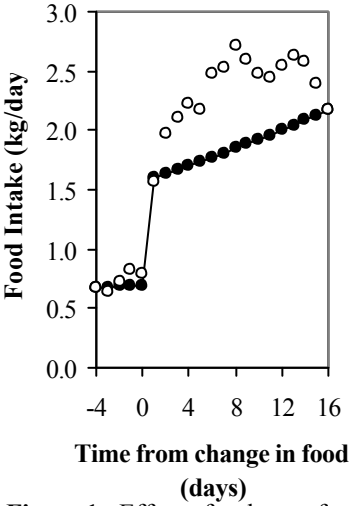


**Figure 1**. Effect of a change from 70% SBP to 70% WB on the measured - ?, and predicted - ● values of food intake.

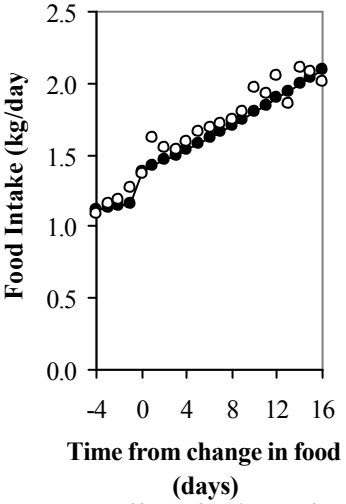


**Figure 2**. Effect of a change from 97% WB to 48% WB on the measured - ?, and predicted - ● values of food intake.

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Whittemore E.C., Kyriazakis I., Tolkamp B. and Emmans G.C. 2001. The short term feeding behavior of growing pigs fed foods differing in bulk content. *Physiology and Behavior* (submitted).

# Description and validation of a harmonised model of the growing pig for the optimisation of the utilisation and excretion of nutrients

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**Introduction** The proposed model may be considered as an advance upon previous models; giving a synthesis of the contemporary information base and having a structure with maximum flexibility and minimum usage of fixed coefficients. It is distinctive in that it includes: (1) linkage from dietary nutrients to tissue retention via algorithms for main-stream biochemical processes; (2) a unified driver allowing compatible calculation of (i) the nutrient costs of energy and of protein cycling, (ii) the nutrient costs of maintenance and protein retention, and (iii) the interactions between energy and protein usage; (3) prediction of the rate and composition of tissue retention during growth, and of the cost of thermoregulation; (4) reversal of the role of conventional model parameters such that they become out-turns, rather than inputs, of the model, including (i) the metabolisability of energy, (ii) the ideality of protein and the efficiency of use of ileal digested ideal protein, (iii) the efficiency of utilisation of energy (k) for maintenance ($k_M$), for protein ($k_{Pr}$) and lipid ($k_{Lr}$) retention, which are accepted as variables derived from calculation of efficiencies of production of ATP.

**Materials and methods** The elements are largely a synthesis of algorithms presented by Whittemore *et al.* (2001a, b, c), which also suggested proofs of principle. Nutrient intake is defined in terms of starch, non-starch polysaccharide, amino acids, oil and ash. The products of fore gut digestion are ileal digested starch, true ileal digested amino acids, and ileal digested lipid, which are absorbed to yield usable glucose, amino acids and lipids. Hind gut digestion yields VFA, gaseous losses and faecal excretions. Amino acids are prioritised to body protein retention, turnover and excretion; lipids are prioritised to lipid retention; while energy-containing end-products of digestion not having contributed carbon skeletons are catabolised, yielding ATP to drive tissue synthesis, support and turnover. Chemical masses are accumulated into carcass tissues and whole-body live weight by use of experimentally-derived empirical relationships. Other routines attempt quantification of the influence of climatic environment, disease and activity.

**Results** The efficiency of use of ME for protein retention ($k_{Pr}$) was found to fall from 0.55 at 5 kg pig protein mass to 0.45 at 15 kg protein mass. The maximum efficiency of use of ileal (apparently) digested ideal protein was found to be 0.86. The gross out-turns from the model were tested against data from a recently completed experiment involving 104 pigs of three types grown from 25 to 115 kg live weight and slaughtered serially for carcass and chemical analysis following daily measurement of live weight and feed intake. Model results for individual pigs were regressed upon those observed. Effective simulation is shown by target values of $y_0 = 0$ and $b = 1$ in the equations;

Modelled lipid mass = 1.95 (± 0.275) + 0.829 (± 0.0197)•Observed lipid mass $r^2 = 0.95$
Modelled protein mass = –1.05 (± 0.364) + 1.13 (± 0.027)•Observed protein mass $r^2 = 0.94$
Modelled live weight = – 1.96 (± 1.31) + 1.05 (± 0.017)•Observed live weight $r^2 = 0.97$
Modelled P2 backfat depth = 3.50 (± 0.275) + 0.663 (± 0.0197)•Observed P2 $r^2 = 0.76$

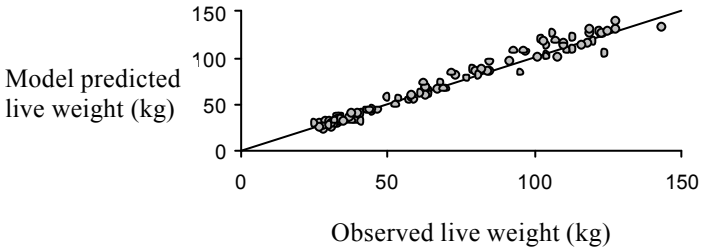


Figure 1. Chart of model predicted live weight versus observed live weight for the 104 pigs. The solid line represents the 1:1 relationship

**Conclusion** This model will increase the likelihood of optimising diverse production systems with regard to nutrient use and pollution control. The model will be used at the heart of an Integrated Management System for pig production that will further incorporate aspects of iteration, learning and diagnosis. The simulation of P2 backfat depth requires further sophistication, and appears sensitive to pig type.

**Acknowledgements** The work is part of the UK DEFRA LINK Sustainable Livestock Production Programme Project LK0614 *Integrated Management Systems for Pig Nutrition Control and Pollution reduction*. The support of the sponsors; DEFRA, MLC, BOCM Pauls Ltd, PIC Ltd, and Osborne Ltd is gratefully acknowledged.

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# The short term feeding behaviour of pigs given access to foods differing in bulk content

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**Introduction** We need to improve our understanding of the factors that are important for the control of food intake on high bulk foods. The study of short term feeding behaviour (STFB) may help to do this. The objective of this experiment was to study the effects of giving foods differing in bulk content on the STFB of growing pigs. It was expected that the foods would result in different levels of daily intake and that this would be reflected as differences in STFB between the foods. Two hypotheses were developed based on ideas about the way in which a physical constraint to intake could arise. $H_1$; there would be less diurnal variation in feeding on high bulk foods that limit intake. $H_2$; feeding patterns on bulky foods would be less flexible than those on a control food when feeding time is limited by reducing time of access to the feeder.

**Materials and methods** Sixteen entire male commercial hybrid pigs with an average weight of 14.3 (s.d.1.09) kg were placed into individual pens, each with a computerised feeder. Three foods, fed *ad libitum*, were used. C was a control food based on micronised wheat with 13.2MJ DE and 243g CP per kg fresh food. The high bulk foods were based on either 70% sugar beet pulp (SBP) with 11.1MJ DE and 206g CP per kg fresh food or 70% wheatbran (WB) with 10.0MJ DE and 184g CP per kg fresh food. The experiment consisted of 4 experimental periods and 6 treatment groups. Periods 1 and 2 were 21 days each and represented a change over design, here pigs were changed from C to either WB or SBP, from WB to C or SBP, or from SBP to C or WB (test of H1). In Period 3 the pigs remained on the same food as in Period 2, but had access to food for 6 hrs a day (from 10:00 to 16:00 hrs, test of H2). In Period 4 the pigs were returned to 24 hrs access to food, this took account of any changes in STFB occurring as a result of the pigs growing. Periods 3 and 4 were each 10 days. Intake was recorded daily and live weight twice a week. Data on intake, gain and STFB were analysed using ANOVA. Food and treatment were used as the factors in the model. The block structure in the model was for individual pigs nested within time. The results from Period 3 were compared with the average of Periods 2 and 4.

**Results** There were significant differences in STFB between the foods. Pigs fed WB and SBP spent longer eating ($P < 0.001$) and had slower feeding rates ($P < 0.001$) than pigs fed C. $H_1$ was rejected, as there was no difference in diurnal variation between the foods. Feeding was not extended in to the night on WB and SBP and there was no difference in the proportion of feeding that occurred during the night between the foods (Figure 1). $H_2$ was supported, on both WB and SBP intake ($P < 0.001$) and LWG ($P < 0.001$) were significantly reduced in Period 3. There was a reduction in the number of visits made to the feeder in a day on all three foods in Period 3 (at most $P = 0.01$). Pigs fed C increased intake/visit ($P < 0.05$) and feeding rate (NS) when time of access was reduced, but there was no effect of reducing length of access on intake/visit or feeding rate of pigs fed WB or SBP.

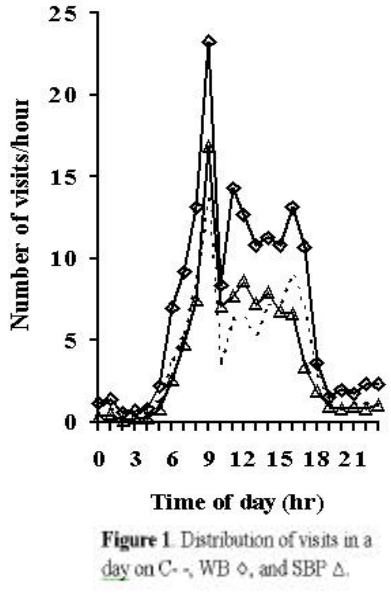


**Figure 1**. Distribution of visits in a day on C- -, WB ◇, and SBP △.

**Table 1**. Effect of reducing time of access to the feeder on the STFB of pigs fed one of three foods differing in bulk content.

| Food | Period | Median Visit Duration (secs) | Visits/day | Intake/visit (g) | Feeding rate (g min$^{-1}$) |
|---|---|---|---|---|---|
| C | Average 2+4 | 30.0 | 102.0 | 25.0 | 42.0 |
| | 3 | 40.0 | 72.0 | 46.0 | 48.0 |
| | s.e.d. | 10.8 | 9.50 | 9.70 | 3.00 |
| WB | Average 2+4 | 26.0 | 189.0 | 16.0 | 35.0 |
| | 3 | 23.0 | 151.0 | 18.0 | 33.0 |
| | s.e.d. | 6.20 | 5.50 | 5.60 | 1.70 |
| SBP | Average 2+4 | 56.0 | 109.0 | 23.0 | 16.0 |
| | 3 | 79.0 | 67.0 | 29.0 | 15.0 |
| | s.e.d. | 6.20 | 5.50 | 5.60 | 1.70 |
| s.e.d. for between | | 15.6 | 27.3 | 10.6 | 4.50 |
| Significance | | | | | |
| Food | | * | ** | NS | *** |
| Period | | * | *** | NS | NS |
| Food*Period | | NS | NS | NS | NS |

**Conclusions** The foods used differed in bulk content and affected STFB. The differences in STFB were consistent, in part, with those expected to occur if a bulk constraint operates. Contrary to expectation, a higher bulk content had no significant effect on the diurnal intake pattern of pigs; the reason for this and its physiological basis remain unclear. Pigs fed the high bulk foods did not maintain intake and performance when time of access to the feeder was reduced, mainly due to the absence of any adaptive change in STFB. It is argued that this could be the result of a physical limitation to intake.

## The influence of grass height on bite dimensions of horses.

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**Introduction** The management and maintenance of swards grazed by horses is known to be a difficult task. In a short period of time (2 to 3 weeks) pastures used for horse grazing develop patches of bare ground, short grass and areas which horses refuse to graze because of faecal contamination. Bite depth and bite area are important in determining the effects of grazing on the vegetation. The dimensions of the bite have been investigated in cattle (Ungar *et al*., 1999), sheep and goats (Concha, 2000), but not horses. The interaction of the horse with the pasture surface is poorly understood but important to improve management and maintenance of areas grazed by horses. The study reported examines the influence of grass height on bite dimensions, i.e. bite depth, bite volume and bite area of the horse at grazing.

**Material and methods** The experimental design was a modification of Laca *et al*. (1992). Perennial rye-grass (*Lolium perenne*) micro-swards of known tiller density (0.3 tillers/$cm^2$) were grown on rockwool substrate (40 x 90 cm). Four sward heights (3, 8, 15 and 19 cm) were maintained by cutting and offered to horses. Prior to offer the biomass ($g/m^2$) and bulk density ($g/m^3$) were determined for each height from ungrazed microswards of identical sward height to those grazed. Eight horses (Thoroughbred X; 512 kg, se 24.7; 2-4 years of age) were used in the grazing study. The horses were offered the microsward until six bites were recorded. Bite depth was calculated by measuring the difference between previous sward height and residual height of the area grazed. Individual bite weight was estimated by the difference between the pre-grazing biomass and the residual biomass. As bite weight is the product of bite volume and bulk density of the grazed horizon (Laca *et al.,* 1992), thus bite volume was calculated. Differences in bite weight, depth and volume were assessed using analysis of variance.

**Results** Bite depth, weight, area and bite volume in relation to sward height are given in Table 1. Bite depth increased linearly with sward height, whereas bite weight and volume of the horses increased quadratically with sward height. The effect of sward height on bite dimension was significant for all variables measured ($p<0,05$), but there was no significant difference of the effect of sward height on bite area for 3, 8 and 15 cm height ($p<0,07$). The ratio of bite depth to sward height increased with increasing sward height until the sward reached 15 cm. No difference in the ratio of bite depth to sward height between 15 and 19cm swards was observed.

**Table 1**. Effect of grass height on bite depth, weight, volume and area.

| Mean grass height (cm) | s.d. | Bite depth (cm) | s.d. | Bite weight (g) | s.d. | Bite volume ($cm^3$) | s.d. | Bite area ($cm^2$) | s.d. |
|---|---|---|---|---|---|---|---|---|---|
| 3 | 0.10 | 1.72 | 0.17 | 0.64 | 0.13 | 112.83 | 22.94 | 65.80 | 11.35 |
| 8 | 0.21 | 4.99 | 0.46 | 1.82 | 0.17 | 302.71 | 28.55 | 61.36 | 10.05 |
| 15 | 0.36 | 9.91 | 0.59 | 4.16 | 0.78 | 736.97 | 126.25 | 74.31 | 11.34 |
| 19 | 0.23 | 12.58 | 1.09 | 6.74 | 1.13 | 1333.95 | 257.53 | 107.88 | 29.09 |

**Discussion** Bite depth, weight, area and volume of the horses were not a fixed size, but adjusted to sward height. With an increase in sward height the horses increased bite depth, weight and volume. However of bite area did not change significantly for the heights 3, 8, and 15 cm. The grazing behaviour of horses in response to different grass heights cannot be solely explained by an energy-maximised foraging strategy. The response may be influenced by more complex factors for instance the location of plant components in the sward or dietary experience.

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# The effect of sugar-beet pulp on the nutritive value of high-temperature dried alfalfa for ponies

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**Introduction** Feeding horses high levels of cereal starch can result in diet-related azoturia, laminitis and colic, whereas high fibre, forage-based diets do not generally elicit these conditions. Therefore, it would be advantageous to develop fibrous feeds with increased digestibilities, permitting horses with high energy demands to be sustained on greater forage: cereal starch ratios. High temperature dried (HT) alfalfa has been fed to horses for a number of years and it is common practise to combine this with sugar beet pulp (SB) another nutritious fibrous feed for horses. Synergistic effects of SB when added to fibre-based diets have been observed in other species *in vivo* (Longland *et al*., 1994) whereby the digestibility of graminaceous feeds has been increased. However, such effects have been little examined in horses fed a leguminous-forage diet. The aim of this study therefore, was to determine if SB enhanced the digestibility of alfalfa, a forage legume that is increasingly being fed to equines in the UK.

**Materials and Methods** A replicated 4 X 4 Latin square changeover design experiment was used to evaluate the effect of three levels of sugar-beet pulp inclusion on the nutritive value of high-temperature dried alfalfa for ponies. Seven mature Welsh-cross pony geldings and one Welsh-cross pony stallion (280 kg LW s.e. 17.6) were individually housed and were fed each diet at 1.75% dry matter (DM) of bodyweight in two equal meals per day at 12-hour intervals. The experiment consisted of 4 diets; a control (no SB) and three levels of sugar-beet pulp inclusion (100, 200 and 300g SB $kg^{-1}$ DM: diets [SB10], [SB20] and [SB30] respectively). Each experimental period consisted of a 10 day adaptation phase and a five day recording phase when *in vivo* apparent digestibilities of DM (DMD), crude protein (CPD), acid detergent fibre (ADFD), neutral detergent fibre (NDFD) and non-starch polysaccharide (NSPD) were determined. Data were analysed using Latin Square ANOVA (Genstat 5, 2000). Values for SB as a sole feed were calculated by difference.

**Results** *In vivo* apparent digestibility values for each of the experimental diets and SB as a sole feed are shown in Tables 1 and 2 respectively. Inclusion of SB in alfalfa diets had no effect on digestibility of CP, but increased the digestibility of dietary dry matter and cell wall fractions. From Table 2 it appears that inclusion of SB in HT alfalfa diets increased the digestibility of the alfalfa cell wall fraction as the calculated NSP digestibility of SB was usually in excess of 1.

**Table 1**: *In vivo* apparent digestibility of nutrients (g $kg^{-1}$) of the experimental diets

| | DC | SB10 | SB20 | SB30 | s.e.d. | Sig. |
|---|---|---|---|---|---|---|
| DMD | 572.0$^{a}$ | 600.9 $^{b}$ | 618.1$^{bc}$ | 634.0$^{c}$ | 11.29 | *** |
| CPD | 656.5 | 655.3 | 643.2 | 645.3 | 14.82 | NS |
| ADFD | 453.4$^{a}$ | 480.0$^{a}$ | 477.0$^{a}$ | 524.9$^{b}$ | 14.80 | *** |
| NDFD | 474.8$^{a}$ | 517.4$^{b}$ | 533.3$^{b}$ | 586.8$^{c}$ | 13.57 | *** |
| NSPD | 561.6a | 617.8$^{b}$ | 638.8$^{b}$ | 700.1$^{c}$ | 13.80 | *** |

Values in rows not sharing common superscripts differ significantly ($P<0.05$)

**Table 2**: *In vivo* apparent digestibility values (g $kg^{-1}$) for sugar-beet pulp calculated by difference

| | SB10 | s.e. | SB20 | s.e. | SB30 | s.e. |
|---|---|---|---|---|---|---|
| DMD | 861.1 | 172.71 | 798.1 | 48.21 | 778.6 | 45.83 |
| CPD | 644.2 | 176.30 | 581.3 | 78.47 | 619.1 | 57.44 |
| ADFD | 719.6 | 200.39 | 564.1 | 55.24 | 691.7 | 57.21 |
| NDFD | 901.4 | 198.17 | 760.0 | 39.45 | 848.2 | 48.76 |
| NSPD | 1123.7 | 190.58 | 939.6 | 67.82 | 1023.2 | 41.54 |

**Conclusion** It is clear that both HT alfalfa and SB were well digested by ponies and may well be effective replacements for grass hay, which typically has a digestibility of between 0.3 to 0.4 in equids. It appears that the inclusion of SB upgraded the nutritive value of the alfalfa through enhanced digestibility of the alfalfa cell walls.

**Acknowledgements** this study was funded by the BBSRC and Dengie Crops Ltd.

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# Appetency and preference in horses offered lucerne or chalk as a source of calcium

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**Introduction** Lucerne and chalk are sources of calcium used to supplement horses diets. The voluntary ingestion of lucerne varies with its form (Cuddeford, 1994). The objectives of this study were to compare the appetency for different sources of calcium, measured by kinetic of ingestion and selection behaviour (sorting, refusal) and to evaluate the effect of those different sources of calcium supplements on the preference of diets offered as a simultaneous choice. The sources of calcium studied were chalk and dehydrated lucerne presented in three forms: 6 mm diameter pellets, 18 mm diameter pellets and ground.

**Material and Methods** For appetency assessment, the kinetic of ingestion (min/kg), extend of refusal (none, some, lots) and intensity of sorting behaviour (none, some, lots) were measured from 19 privately owned horses in a randomised complete block design. The live weight of the horses ranged from 200 kg to 900 kg. The horses were fed their usual diet, which was either a pelletised (n=7) or all-mash (n=12) concentrate with varied quantities of molasses (none, few, lots). For the measurement, either the usual diet was supplemented with chalk at a level of 8.5 g/kg diet, or an amount of 0.15 of the usual diet was replaced by dehydrated lucerne. The total duration of ingestion of a meal was recorded for each of the four sources of calcium supplement.
For preference assessment, three other horses were used to evaluate the effect of the four calcium supplements in cereal-based diet. The offered diets were made of rolled barley, rolled oats and flaked maize in equal proportions, molasses (30g/kg), vitamin-mineral mixture (10g/kg), mixed either with chalk (13g/kg) or dehydrated lucerne (240g/kg). The horses were trained to consume each diet during 35 days. For the preference trial, the horses received two diets simultaneously, 1 kg in each side of the manger. All combinations of diets, as six blocks, were offered in left and right sides of the manger in randomised complete block design. The factors measured were the kinetic of ingestion of each diet (min/kg), the weight of refusals of each diet (g) and the number of side changes of the horse. Results were treated by analysis of variance and presented as least square means. Means were further compared by student *t* test.

**Results** There was no block effect on the appetency assay (table 1). A first model ($P<0.001$; $r^2=0.935$; table 2) showed a significant effect of calcium supplement ($P<0.05$), quantity of refusals ($P<0.001$) and horse ($P<0.001$) on kinetic of ingestion. The chalk supplemented diet was eaten the fastest and the ground lucerne supplemented diet was eaten the slowest. Horses without refusal ate significantly faster than horses with some and lots of refusal ($P<0.001$). The horse effect was subdivided in a second model ($P<0.001$; $r^2=0.756$; table 2). The effect of refusals was confirmed ($P<0.001$); there was also an effect of the form of the usual diet ($P<0.01$) and of the live weight of the horses ($P<0.001$). Horses accustomed to a pelletised diet ate significantly faster than horses on an all-mash diet. However, no interaction could be shown between calcium supplement and form of usual diet ($P>0.05$). Heavier horses ate faster ($P<0.001$). There were no effect of calcium supplement, sorting behaviour and level of molasses on the kinetic of ingestion ($P>0.05$).

**Table 1** Appetency and preference measured by mean kinetics of ingestion, refusals and changes of selected diet

| | chalk | lucerne 6 mm | ground lucerne | lucerne 18 mm | s. e. m. | P |
|---|---|---|---|---|---|---|
| Appetency assessment: | | | | | | |
| Kinetic of ingestion (min/kg) | 10.26 | 12.34 | 15.28 | 14.66 | 2.957 | NS |
| Preference assessment: | | | | | | |
| Kinetic of ingestion (min/kg) | 8.21 [a] | 8.66 [a] | 11.61 [b] | 9.19 [a] | 0.628 | ** |
| Refusals (g) | 24 [a] | 18 [a] | 278 [b] | 24 [a] | 33.2 | ** |
| Side changes (n) | 14.5 | 15.0 | 17.1 | 15.4 | 1.87 | NS |

Within row, means with a different letter differ significantly ($P<0.01$)

Results of the preference trial were adjusted for the significant horse effect ($P<0.01$) and are presented in table 1. There were neither block nor side of manger effects. The calcium supplement influenced the kinetic of ingestion ($P<0.01$) and the quantity of refusals ($P<0.01$). The kinetic of ingestion was slower and the refusals were larger for the ground lucerne supplemented diet than for any other supplemented diets. The number of side changes was not significantly influenced by the calcium supplements.

**Table 2** Variance of appetency (kinetic of ingestion)

| | first model | | second model | |
|---|---|---|---|---|
| | df | P | df | P |
| calcium supplement | 3 | * | 3 | NS |
| refusals | 2 | *** | 2 | *** |
| horse | 18 | *** | | - |
| live weight | | - | 1 | *** |
| sorting behaviour | | - | 2 | NS |
| form of usual diet | | - | 1 | ** |
| quantity of molasses | | - | 2 | NS |
| model | 23 | *** | 11 | *** |
| error | 52 | | 64 | |

**Conclusions** In terms of appetency and preference, chalk appeared as the most appreciated calcium supplement, while ground lucerne was the least appreciated as shown by the high refusals and the long time taken to ingest the diet.

**Reference** Cuddeford D. 1994. Artificially dehydrated lucerne for horses. *The Veterinary Record.* **135**: 18, 426-42

# Ingestion and metabolic profile in horses offered lucerne or chalk as a source of calcium

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**Introduction** When horses are on diets that are predominant in cereals, the combination of low concentrations of calcium in the diet and the binding of calcium by phytates may result in inadequate calcium intake (Rose, 1990). Chalk and dehydrated lucerne are rich in calcium. It has been shown that the voluntary ingestion of lucerne varies with its form (Cuddeford, 1994). The aim of this study was to evaluate the effect of different sources of calcium (chalk *vs* dehydrated lucerne) and lucerne forms (6 mm diameter pellets, 18 mm diameter pellets and ground lucerne) on the ingestion and on the metabolic profile in horses offered a cereal-based diet.

**Material and Methods** Four horses were used in a ramdomized 4x4 Latin square design with four blocks of 28 days, separated by 7 days of transition. The horses were kept at a constant live weight, and fed twice daily a concentrate diet made of rolled barley, rolled oats and flaked maize in equal proportions, molasses (30g/kg), vitamin-mineral mixture (10g/kg), mixed either with chalk (13g/kg) or dehydrated lucerne (240g/kg) according to treatment. Straw was fed *ad libitum.* The horses on lucerne-supplemented diet were offered 1,33 more concentrate. The duration of ingestion of 3 kg of concentrate was recorded. Blood was collected from the jugular vein on days 21 and 28 of each period. The measured plasma metabolites were total calcium (Ca), ionised calcium ($Ca^{++}$), inorganic phosphorus (P), 25-hydroxyvitamin D (25-OHD), hydroxyproline (Hyp), total alkaline phosphatase (TALP), bone alkaline phosphatase (BALP) and osteocalcin (OC). Data were subjected to least-square analysis of variance, with treatment means further compared by *t* test.

**Results** No block effect was encountered on the ingestion parameters. The ingestion results in table 1 are adjusted for the significant horse effect ($P<0.05$). Some refusals of ground lucerne were observed. The ingestion of concentrate was, as expected, lower for the chalk-supplemented than for the lucerne-supplemented diets ($P<0.01$). Calcium and phosphorus intakes were also lower for the chalk-supplemented than for the lucerne-supplemented diets ($P<0.01$). The calcium:phosphorus ratio of all diets was between 1.6 and 1.8, values considered as adequate. The effect of calcium source and lucerne presentation on the ingestion of straw and on the duration of concentrate ingestion was not significant, although the mean duration of ingestion appeared lower for the chalk-supplemented concentrate, and higher for the ground lucerne supplemented concentrate.

**Table 1** Effect of calcium supplement on daily ingestion weighed for the horse effect, and plasma metabolites.

| | chalk mean | lucerne 6 mm mean | ground lucerne mean | lucerne 18 mm mean | s. e. | sign. P |
|---|---|---|---|---|---|---|
| Ingestion: | | | | | | |
| Concentrate (kg DM/d) | 4.85[a] | 6.58[b] | 6.08[b] | 6.34[b] | 0.239 | ** |
| Straw (kg DM/d) | 2.98 | 2.61 | 2.84 | 2.70 | 0.151 | NS |
| Calcium (g/d) | 35.0[a] | 45.9[b] | 43.3[b] | 44.4[b] | 1.36 | ** |
| Phosphorus (g/d) | 20.9[a] | 26.2[b] | 24.2[b] | 25.6[b] | 0.80 | ** |
| Duration of ingestion: | | | | | | |
| Concentrate (min/3 kg) | 27.5 | 32.5 | 37.3 | 32.3 | 2.98 | NS |
| Plasma metabolites: | | | | | | |
| Total calcium (mg/l) | 108.5 | 103.5 | 110.0 | 109.5 | 2.14 | NS |
| Ionised calcium (mg/l) | 53.4 | 51.6 | 41.8 | 51.6 | 4.93 | NS |
| Inorganic phosphorus (mg/dl) | 4.2 | 4.0 | 4.1 | 4.2 | 0.13 | NS |
| 25-OHD (ng/ml) | 2.3 | 2.2 | 1.0 | 1.2 | 0.44 | NS |
| Hydroxyproline (mg/l) | 0.43 | 0.48 | 0.50 | 0.48 | 0.053 | NS |
| TALP (U/l) | 200.5 | 193.9 | 205.0 | 210.5 | 6.00 | NS |
| BALP (ng/l) | 29.86 | 28.99 | 31.40 | 31.54 | 1.179 | NS |
| Osteocalcin (ng/ml) | 7.63 | 8.22 | 8.73 | 7.72 | 0.624 | NS |

Within row, means with a different letter differ significantly ($P<0.05$)

The effect of calcium source and lucerne presentation on the measured plasma metabolites were not significantly different, which was expected since the four diets were adequately balanced in calcium and phosphorus. There was a block effect on Ca, $Ca^{++}$, P, TALP and BALP; there was a horse effect on TALP, BALP and OC, and there was a blood sample effect on $Ca^{++}$ and Hyp. Results in table 1 were adjusted for those significant effects.

**Conclusions** The lower calcium and phosphorus intakes of the chalk-supplemented diet had no significant effect on the measured metabolites in the profile. Chalk, 6 mm diameter lucerne pellets, 18 mm diameter lucerne pellets and ground lucerne are equivalent calcium supplements when mixed to a cereal based diet.

**References** Cuddeford D. 1994. Artificially dehydrated lucerne for horses. *The Veterinary Record.***135**: 18, 426-429
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# Voluntary feed intake, apparent digestibilities and nutritive values in ponies given *ad libitum* access to complete pelleted diets containing different levels of unmolassed sugar beet pulp

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**Introduction** Previous work has indicated that unmolassed sugar beet pulp (USBP) may suppress voluntary feed intake (VFI) in ponies when offered as the major component; but not when offered as a relatively minor component of the diet (Hyslop *et al*, 1998, 1999). However, critical levels of USBP inclusion in equine diets remain to be established. This study examines VFI, *in vivo* apparent digestibilities and nutritive values in ponies offered pelleted complete diets containing USBP at inclusion levels between 0 – 800 g/kg dry matter (DM).

**Materials and methods** 6 mature Welsh-cross pony geldings (mean LW 296 kg) were individually housed and offered one of 6 pelleted complete diets *ad libitum*. No other feed was offered. Diets were formulated from dried grass (DG), USBP, minerals and molasses such that USBP inclusion levels were 0, 160, 320, 480, 640 and 800 g/kg DM respectively in the 6 diets. Feed composition in diets U0, U160, U320, U480, U640 and U800 were as follows:- dry matter (DM) 899, 895, 888, 878, 886, 874 (g/kg); crude protein (CP) 158, 131, 149, 138, 125, 120 (g/kg DM); and neutral detergent fibre (NDF) 527, 547, 516, 498, 485, 490 (g/kg DM). The experiment was a 6 x 6 latin square changeover design lasting for 6 periods of 21 days. Each 21 day period consisted of a 16 day adaptation and a 5 day recording phase when dry matter intake (DMI), *in vivo* apparent digestibilities of DM (DMD), organic matter (OMD), CP (CPD), acid detergent fibre (ADFD), NDF (NDFD) and gross energy (GED) along with digestible energy (DE) and digestible CP (DCP) contents were determined. Differences between diets were determined by analysis of variance. *In vivo* apparent digestibilities, DE and DCP contents for USBP alone were also estimated by linear regression.

**Results** The quadratic relationship between individual pony DMI (g/kg $LW^{0.75}$) and USBP inclusion rate is given in Figure 1. Despite large variation between individual ponies, voluntary DMI declined on average from 7.96 to 5.18 kg/d (Table 1). With the exception of protein digestibility, *in vivo* apparent digestibilities and DE contents generally increased as USBP inclusion level increased (Table 1). Conversely, CPD and DCP content declined. Predicted OMD, ADFD and NDFD for USBP alone were all in excess of 750 g/kg whereas CPD was low at 346 g/kg (Table 2). Consequently, predicted DE content at 12.5 MJ/kg DM was high but predicted DCP content at 37 g/kg DM was low.

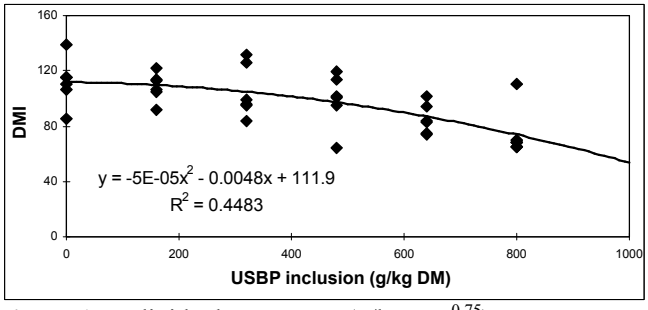


**Figure 1**. Individual pony DMI (g/kg $LW^{0.75}$)

**Table 2**. Digestibilities and nutritive values of USBP.

| (g/kg) | USBP | s.e. | | USBP | s.e. |
|---|---|---|---|---|---|
| DMD | 736 | 17.2 | | | |
| OMD | 776 | 15.8 | DE | 12.5 | 0.36 |
| CPD | 346 | 31.3 | (MJ/kg DM) | | |
| ADFD | 672 | 23.2 | DCP | 37 | 4.02 |
| NDFD | 780 | 31.6 | (g/kg DM) | | |
| GED | 690 | 19.6 | | | |

**Table 1**. Average DMI, *in vivo* apparent digestibilities and nutritive values in complete diets containing USBP.

| | U0 | U160 | U320 | U480 | U640 | U800 | sed | Sig | | U0 | U160 | U320 | U480 | U640 | U800 | sed | Sig |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DMI | | | | | | | | | (g/kg) | | | | | | | | |
| kg/d | 7.96$^{a}$ | 7.74$^{a}$ | 7.46$^{ab}$ | 7.02$^{b}$ | 6.01$^{c}$ | 5.18$^{d}$ | 0.341 | * | DMD | 550$^{ab}$ | 546$^{a}$ | 584$^{b}$ | 643$^{c}$ | 661$^{cd}$ | 698$^{d}$ | 17.6 | * |
| g/kg LW | 27.0$^{a}$ | 26.2$^{ab}$ | 25.4$^{ab}$ | 24.0$^{b}$ | 20.6$^{c}$ | 18.1$^{c}$ | 0.12 | * | OMD | 564$^{a}$ | 570$^{a}$ | 615$^{b}$ | 674$^{c}$ | 697$^{cd}$ | 727$^{d}$ | 17.4 | * |
| g/kg$LW^{0.75}$ | 112$^{a}$ | 108$^{ab}$ | 105$^{ab}$ | 99$^{b}$ | 85$^{c}$ | 74$^{d}$ | 4.95 | * | CPD | 574$^{ab}$ | 461$^{b}$ | 488$^{b}$ | 468$^{b}$ | 392$^{c}$ | 399$^{c}$ | 28.2 | * |
| DE | 10.1$^{a}$ | 10.0$^{a}$ | 10.4$^{a}$ | 11.4$^{b}$ | 11.5$^{b}$ | 11.9$^{b}$ | 0.35 | * | ADFD | 418$^{a}$ | 417$^{a}$ | 476$^{a}$ | 538$^{b}$ | 569$^{bc}$ | 623$^{c}$ | 28.9 | * |
| (MJ/kg DM) | | | | | | | | | NDFD | 459$^{a}$ | 516$^{ab}$ | 569$^{b}$ | 640$^{c}$ | 661$^{cd}$ | 708$^{d}$ | 28.1 | * |
| DCP | 91$^{a}$ | 60$^{b}$ | 73$^{c}$ | 65$^{bc}$ | 49$^{d}$ | 48$^{d}$ | 4.24 | * | GED | 531$^{a}$ | 530$^{a}$ | 557$^{a}$ | 612$^{b}$ | 628$^{bc}$ | 662$^{c}$ | 17.7 | * |
| (g/kg DM) | | | | | | | | | | | | | | | | | |

Values not sharing common superscripts differ significantly (P<0.05).

**Conclusions** Increasing USBP inclusion level from 0 – 800 g/kg DM progressively reduced VFI by 35 %. The critical level of USBP inclusion required before a statistically significant reduction in VFI occurred lay between 320 and 480 g/kg DM. DE content increased but DCP content declined as USBP inclusion increased, reflecting USBP values.

**Acknowledgements** This work was funded by Trident Feeds Ltd.

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# The effect of breed on the expression of adverse social behaviour in pigs

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**Introduction** The performance of tail biting and other harmful social behaviours is a common problem on pig farms. Many risk factors relating to tail biting have been identified, but the problem remains intractable. One contributory factor may be the genetic makeup of pigs but, as with most pig behaviour, there has been little research into the genetic basis of its expression. The aim of the current experiment was to investigate the genetic component of harmful social behaviours, such as tail biting, by assessing breed differences in the predisposition to perform these behaviours.

**Material and Methods** The behaviour of three pig breeds, with 100 pigs per breed [LW - Large White, LR - Landrace, DR - Duroc] was observed in a 'tail chew test' previously indicated as potentially identifying tail and ear biters (Breuer *et al*., 2001) and the performance of harmful social behaviour (HSB) directed to pen mates was observed in barren flat deck pens at four weeks post weaning. Two boars and 2 gilts (1 heavy and 1 1ight) from each litter were selected on the basis of weight at 21 days. The 'tail chew test' involved observing the behaviour of individual pigs towards two pieces of rope over a 10 minute period, and was performed twice at approximately 25 and 26 days of age. When moved to the flat deck pens at 28 days of age, the experimental pigs were put into mixed-breed single-sex groups of 10. At approximately 56 days of age, the occurrence of HSB was recorded over 2 consecutive days (1 hour per day) using a group 'period occurrence' scanning method. The data were combined for the 2 days for both the tail chew test and HSB, and a 2-way analysis of variance was carried out on these data, with breed and gender as factors. A 1-way analysis of variance was also carried out on these data within breed, with sire as the factor. The behaviour of each individual pig in the tail chew test was correlated with the HSB of that pig.

**Results** Breed had a significant effect on rope directed behaviour in the tail chew test and on HSB (Table 1). DR pigs interacted with the ropes in the tail chew test more often and for longer than LW and LR pigs. DR pigs directed more HSB towards pen mates than the other breeds; for example, DRs were observed nosing and ear biting pen mates more often than LR and LW. LR pigs tended to belly nose pen mates more often than other breeds. Significant sire effects within breed on the performance of rope directed behaviour and on HSB were also found. For example, the pigs of one DR sire performed more rope directed behaviours than the pigs from the four other DR sires (70.0 vs 17.5, 22.0, 44.2 and 26.5, observations per 10 minute test, $P<0.001$). There were positive but low correlations between rope directed behaviour and the performance of some HSB; the number of times that behaviour was directed towards the rope was positively correlated with ear biting and tail biting ($r=0.151$, $P<0.01$ and $r=0.120$, $P<0.05$).

Table 1 *The effect of pig breed on the frequency of, and time spent in, rope directed behaviours and on the number of periods (from 20/hour) in which pig-directed harmful social behaviour was observed*

| *Behaviour* | *DR* | *LR* | *LW* | *s.e.d.* | *P value* |
|---|---|---|---|---|---|
| **Rope directed behaviour:** | | | | | |
| Number of observations per 10min test | 26.4[b] | 20.6[a] | 18.8[a] | 1.73 | <0.001 |
| Time spent (s/10 min) | 38.7[b] | 24.1[a] | 26.6[a] | 3.90 | <0.001 |
| **Harmful social behaviour:** | | | | | |
| Nosing pig | 12.9[b] | 10.9[a] | 11.6[a b] | 0.84 | 0.05 |
| Ear biting | 6.5[b] | 4.4[a] | 5.7[a] | 0.53 | <0.001 |
| Belly nosing | 0.9[a] | 1.5[a] | 0.9[a] | 0.29 | 0.06 |

[ab] Means on the same row with same superscript are not significantly different

**Conclusion** The results suggest that the motivation to perform harmful social behaviour may have a genetic link, with DR performing more biting activities, such as ear biting, and more rope directed activities, and LR tending to perform more belly nosing. This indicates that when a pig is subject to challenge (e.g. by early weaning or barren housing), the type of behaviour that it performs may depend on its genetic predisposition. Further evidence of involvement of a genetic component in the expression of harmful social behaviour is supplied by the significant sire effects within breed. An experiment is currently underway to further investigate these sire effects.

**Acknowledgements** The authors gratefully acknowledge funding from DEFRA and the assistance of Rattlerow Farms Ltd and farm staff at Coneywood Farm.

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## An investigation into the effect of tryptophan on tail chewing behaviour of growing pigs

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**Introduction** Previous work (McIntyre and Edwards, 2001) revealed a significant correlation between plasma tryptophan (TRP) concentration and blood preference scores when pigs were fed a low CP diet. Increasing dietary TRP in rats causes increases in plasma and brain TRP, and brain serotonin concentration (Fernstrom and Wurtman, 1971), which appear to be highly localised to serotonin-containing neurones that may monitor the metabolic state and control behaviour. Uptake of TRP at the blood-brain barrier and serotonin level may also be increased by a higher TRP: LNAA (large neutral amino acids) ratio and level of dietary carbohydrate. This study aimed to determine if pigs fed diets differing in TRP and TRP: LNAA ratios differed in their behaviour or in their preference for blood during a model tail test.

**Materials and methods** 40 pigs were selected at 10 weeks of age, weighing 21·9kg (S.D. = 2·0kg). The pigs were divided into 4 groups of 10 and randomly allocated within group and sex to one of 5 dietary treatments. The pigs were individually fed *ad lib*, twice each day, and had free access to water. For the first 2 weeks, all pigs were fed a commercial diet, providing a baseline for the behaviour and model tail tests. The pigs were weighed on a weekly basis, and allocated to the experimental diets according to their weights at the end of week 2. The experimental diets (Table 1) were formulated to provide 14 MJ DE.kg$^{-1}$ and 12 g.kg$^{-1}$ lysine with one of 3 levels of tryptophan, a deficient (0·12%), an optimal (0·22%) and a supplemented level (0·32%), and modified TRP: LNAA ratio or starch content.

**Table 1** Composition of the experimental diets

| | A | B | C | D | E |
|---|---|---|---|---|---|
| **Tryptophan (%)** | **0.12** | **0.22** | **0.22** | **0.22** | **0.32** |
| LNAA | 4.89 | 4.98 | 4.74 | 4.68 | 4.78 |
| **TRP:LNAA** | **0.03** | **0.04** | **0.05** | **0.05** | **0.07** |
| Starch (%) | 35.00 | 34.94 | 35.35 | 45.00 | 44.94 |

The behaviour of all pigs in each pen was time sampled at two-minute intervals, for two, 20-minute sessions each day, for 5 days during weeks 2, 4, 6, 9 and 11 of the experiment. The pigs were also subjected to the model tail test for 3 days during weeks 1 and 7, by presenting them with 2 tail models made of sash cord on which they could chew. The tail models were soaked overnight in either whole pig's blood or distilled water, and the animals' chewing behaviour and preference was directly observed and recorded every 6 seconds, for 24 minutes (McIntyre and Edwards, 2001). Data were meaned across weeks for each pig and analysed by ANOVA with treatment and pen as factors.

**Results** Dietary treatment had a significant effect on the blood preference scores in week 7, with the pigs fed Diet A having the greatest preference for the blood-soaked tail model (Table 2). These pigs also spent significantly less time sleeping, and significantly more time nosing their pen, pen-mates and bedding than those fed the other dietary treatments. Diet A pigs also engaged in more pig-directed behaviours, although this difference was not significant. Over the 11-week period the pigs on Diet A had lower weight gain (Table 2).

**Table 2** Behaviour and performance of the experimental animals

| Mean frequency of behaviours per session | A | B | C | D | E | s.e.m. | P |
|---|---|---|---|---|---|---|---|
| Sleeping | 4·425$^{a}$ | 4·782$^{a,b}$ | 5·217$^{a,b}$ | 4·955$^{a,b}$ | 5·573$^{b}$ | 0·215 | ** |
| Nosing straw, pig & pen | 3·818$^{a}$ | 3·083$^{a,b}$ | 2·913$^{b}$ | 2·965$^{a,b}$ | 2·618$^{b}$ | 0·215 | ** |
| Straw-directed behaviours | 2·408$^{a}$ | 2·232$^{a}$ | 1·875$^{a,b}$ | 2·120$^{a,b}$ | 1·563$^{b}$ | 0·149 | ** |
| Pig-directed behaviours | 0·650 | 0·550 | 0·445 | 0·492 | 0·537 | 0·529 | NS |
| Blood preference score (wk 7) | 0·655$^{a}$ | 0·516$^{a,b}$ | 0·462$^{b}$ | 0·590$^{a,b}$ | 0·548$^{a,b}$ | 0·042 | * |
| Liveweight gains (kg.d$^{-1}$) | 0·549$^{a}$ | 0·733$^{b}$ | 0·748$^{b}$ | 0·780$^{b}$ | 0·812$^{b}$ | 0·028 | *** |

Means within rows with different superscripts are significantly different (P<0·05)

**Conclusion** Those pigs fed Diet A with low TRP, low dietary TRP: LNAA and low starch content had the greatest preference for blood, as demonstrated by the model tail test. Those pigs fed diets with excess TRP were the least active, and spent the most time sleeping. Increasing dietary TRP in pig feed might reduce exploratory activity and possibly reduce incidences of tail biting.

**Acknowledgements** This work forms part of a PhD studentship funded by MLC.

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# Consistency in piglet mortality in individual sows and factors affecting piglet mortality

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**Introduction** Pre-weaning mortality (11.9% as estimated by M.L.C., 2000) continues to be a major economic and welfare problem in commercial indoor pig production. The main causes of mortality are crushing by the sow and low viability/starvation. Both of these causes of death may be as a result of increasing sow body size and smaller piglet body size as a result of intense genetic selection for increasing litter size. However it is unclear whether sows are consistent across parities in their level of pre-weaning mortality. Therefore this study aimed to examine individual differences in piglet mortality of sows throughout their reproductive life, investigate factors likely to affect piglet mortality, and to assess whether piglet mortality may be a candidate trait for genetic selection.

**Materials and methods** Farrowing records were collected for 125 Large White x Landrace sows on a commercial pig unit (using farrowing crates) across a period of 11 years. All of the 125 sows had farrowing records from their first 4 parities at least, however some sows had farrowing records up to parity 9 resulting in a total of 793 farrowing records. If a farrowing record was missing (lost record card) it was entered as missing data. Data recorded were piglet births, deaths, gestation length, season and litter birth weight. GLMM (Generalised Linear Mixed Model) was used to analyse the following causes of death: crushing, low viability (LV) and total deaths (total) (including disease, abnormalities) expressed as a proportion of the number born alive, and stillborns expressed as a proportion of the total piglets born (alive and dead). Two random effects were fitted: sow and parity. Fixed effects were: born alive (total piglets born for stillborn), litter birthweight, gestation length, season and parity. Estimated variance components were used to assess whether significant variation between sows existed (using a z-test) and to assess effects of terms in the fixed model we used the coefficients of effects for direction and the Wald statistic for significance level (based on a Chi-square distribution).

**Results** The total number of piglets born was 8267, out of which 540 were intra-partum stillbirths (stillbirth rate 6.5%). Out of the 7727 piglets born alive, 1185 died before weaning resulting in a mortality rate of 15.3% (total deaths). The most common causes of liveborn piglet deaths were crushing (48%) and low viability/starvation (31%). There was large variation between sows in the mean (± s.d) percentage of piglets dying per litter as a result of the different causes of mortality: crushing 6.5% (±5.0), LV 4.6% (±4.6), total 14.4% (±8.1) and stillborn 6.0% (±4.1). This variation between sows (over and above within sow variation) was found to be significant in all causes of mortality suggesting that sows are consistent in piglet mortality (Table 1). The repeatability estimates (R) were typical for this type of trait (Table 1). All causes of mortality, except LV, increased with parity. Gestation length and season did not affect piglet mortality. All causes of mortality increased as the number of piglets born alive increased and as litter weight decreased (Table 2).

**Table 1** Variation between sows in piglet mortality and repeatability estimates (R).

| | Variance component | | *P-value* | *R* |
|---|---|---|---|---|
| | **Estimate** | **s.e.** | | |
| Crushing | 0.191 | 0.0662 | <0.01 | 0.163 |
| LV | 0.194 | 0.0960 | <0.05 | 0.120 |
| Total | 0.086 | 0.0380 | <0.05 | 0.063 |
| Stillborn | 0.203 | 0.0643 | <0.001 | 0.276 |

**Table 2** The coefficients of effects of terms in the fixed model on different types of piglet mortality.

| | Crushing | LV | Total | Stillborn |
|---|---|---|---|---|
| Born alive | $0.30^{***}$ | $0.22^{***}$ | $0.25^{***}$ | - |
| Total piglets born | - | - | - | $0.25^{***}$ |
| Litter birthweight | $-0.23^{***}$ | $-0.16^{***}$ | $-0.18^{***}$ | $-0.18^{***}$ |
| Parity | ** | n.s. | *** | * |

**Conclusions** This study confirms that crushing and low viability are the major causes of pre-weaning mortality in farrowing crates. It is suggested that the high incidence of crushing and low viability deaths are partly due to genetic selection for litter size as we have found direct evidence that piglet mortality increases with litter size and decreasing litter birth weight.

We also found that sows showed some consistency in all causes of piglet mortality throughout their reproductive life. The variation seen in the causes of piglet mortality is high making it a potential candidate for selection to improve piglet survivability. The repeatability estimates are similar to those seen for survival traits and litter size, however as this is somewhat low, selection response would be improved by selecting on performance over several litters. The consistency of sows in piglet mortality seen in this study may be as a result of individual differences in ability to cope with restrictive farrowing accommodation, uterine effects on piglets or quality of maternal behaviour. Irrespective of the underlying causes, the fact that sows show some consistency in piglet mortality makes this a potential candidate for selection to improve piglet survivability.

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# Effect of leaving piglets in the farrowing rooms post-weaning and mixing pre-weaning on piglet performance.

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**Introduction** The stress caused by weaning piglets is known to have a serious detrimental effect causing a growth check, increased aggression leading to skin lesions and reducing future performance potential. Previous work (Allen *et al*., 2000) has shown that mixing piglets pre-weaning can improve post-weaning performance and reduce skin lesions caused by fighting without detrimental affects on pre-weaning performance and behaviour. The aim of this experiment was to assess the effect of changing the post-weaning environment on piglet performance.

**Material and methods** Forty-eight PIC Camborough 15 (Large White x (Landrace x Duroc)) sows and their litters kept in conventional farrowing crates were randomly allocated to a two x two factorial design. The factors were: - piglets mixed at 14 days of age (M) or kept as litters until weaning (L) and relocated at weaning (W) or left in the farrowing pens at weaning for 9 days (F). Each treatment group had three sows and mixing prior to weaning was carried out by the removal of the boards separating each farrowing crate allowing the piglets access to three pens whilst the sows remained confined in the crates. Fostering was carried out within 24 hours and normal management practices followed. Creep feed was available from day 17, weaning took place on day 24 and piglets were relocated to fully slatted accommodation on either day 24 or 33 post partum. Piglets were weighed and lesion scored on days 0, 7, 14, 17, 24, 27, 33, 36, 40 and 47. Lesion scores were assessed using a scoring system from 0-4 dependant on number and severity of lesions. Performance data were analysed by ANOVA using Genstat for Windows.

**Results** There were no significant mixing x relocation interactions at any time on post-weaning daily live weight gain (dlwg; Table 1). However, there was a tendency for LF to have a higher dlwg during the immediate period following relocations on days 37-40 compared with other treatments. A significant effect of relocation at weaning was observed between days 28-33 and 34-46 treatment W (relocated at weaning) having significantly higher dlwg compared with treatment F (left in the farrowing room). A significant effect of relocation was also seen over the entire post-weaning period that follows the same pattern as days 28-46.

**Table 1**: Effect of mixing pre-weaning and a change of environment post-weaning on piglet post-weaning daily live weight gain* (g/day).

| | Treatment | | | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| | MW | MF | LW | LF | s.e.d | Mix | Relocate | Mix x Relocate |
| Day 25-27 | 91 | 27 | 53 | 50 | 34.4 | 0.755 | 0.170 | 0.212 |
| Day 28-33 | 133 | 118 | 144 | 104 | 18.2 | 0.962 | 0.041 | 0.340 |
| Day 34-36 | 326 | 315 | 338 | 264 | 27.4 | 0.335 | 0.034 | 0.111 |
| Day 37-40 | 363 | 332 | 366 | 413 | 31.0 | 0.060 | 0.730 | 0.083 |
| Day 41-47 | 451 | 407 | 441 | 407 | 33.1 | 0.909 | 0.128 | 0.758 |
| Day 25-47 | 290 | 259 | 290 | 265 | 15.0 | 0.769 | 0.011 | 0.750 |

*Adjusted for weaning weight as a covariate

Total body lesion scores were not significantly affected by the mixing x relocation interaction (Figure 1). There was a significant effect of mixing on total body lesion score on day 17 with treatment M (mixed at 14 days) having a higher lesion score than treatment L (2.6 vs. 1.2, respectively, s.e.d 0.29; $P<0.001$). Significant effects were also seen on days 27 (2.6 vs.4.1, M & L respectively, s.e.d 0.36; $P<0.001$) and 33 (1.1 vs. 1.5, M & L respectively, s.e.d 0.18; $P=0.025$) with treatment L (mixed at weaning) having a higher lesion score than treatment M. There was no significant effect of treatment on FCR or average feed intake (AFI) except for days 28-33 when a significant effect of relocation on AFI was observed (0.24 vs. 0.17kg, W & F respectively, s.e.d 0.022; $P=0.01$).

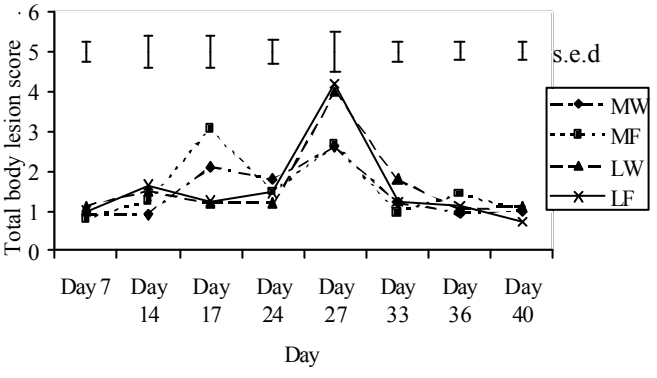


**Figure 1**: Effect of mixing and relocation on total body lesion scores

**Conclusions** The effect of leaving piglets in the farrowing room post-weaning appears to cause a reduction in post-weaning growth and feed intakes. This may be due to the psychological stress of piglets remaining in a familiar environment, without the sow present, being greater than the stress of relocation to a new environment. Mixing piglets pre-weaning reduces lesion scores post-weaning indicating a reduction in aggression after weaning.

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# Effect of weaning age, mixing strategy and group size on the welfare and productivity of weaned pigs

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**Introduction** Reduced welfare and productivity during the post weaning period continues to pose a problem for producers. It may be possible to reduce these problems by altering management practices. The objective of the present study was to investigate the effect of weaning age, mixing strategy and group size on the welfare and productivity of weaned pigs.

**Materials and method** One hundred and ninety-two Large White x Landrace pigs were used in a 2 x 2 x 2 factorial design experiment with two replicates. The factors were age at weaning (4 or 6 weeks), mixing at or prior to weaning (at 3 weeks of age), and number of weaned pigs per pen (6 or 18). All pigs were housed in conventional farrowing crates with slatted floors prior to weaning. From weaning until the end of the experiment at 10 weeks of age the pigs were housed in rooms with supplementary heat and controlled temperature. Space allowance was 0.30, 0.40 and 0.52 $m^2$/pig for pigs at 4, 6 and 8 weeks of age, respectively. Weaned pigs were fed *ad libitum* from single-space feeders supplying both feed and water (one feeder was provided per six pigs). Pens for weaned pigs had solid floors and contained a lying area bedded with peat to a depth of 50 mm and a rack containing straw. The behaviour of focal animals was recorded continuously for 10 minutes twice each week from weaning to 10 weeks of age. Treatment effects on behaviour were assessed during the first 4 weeks after weaning and also between 6 and 10 weeks of age in order to allow for comparison of behaviour between treatments directly after weaning and at a constant age. Injury scores were recorded on a scale of 0 to 4 immediately prior to weaning and 48 hours later. Pigs were weighed individually at 6 and 10 weeks of age. Group means were used as experimental units for all data and treatment effects were assessed by analysis of variance using Genstat 5.

**Results** Effects of weaning age on selected behavioural parameters are listed in Table 1. During the first 4 weeks after weaning pigs weaned at 4 weeks of age spent more time nosing other pigs, head thrusting and fighting, and standing or sitting inactive than pigs weaned at 6 weeks of age ($P<0.05$). Pigs weaned at 6 weeks of age spent more time exploring peat than pigs weaned at 4 weeks of age during the first 4 weeks after weaning ($P<0.01$). Between 6 and 10 weeks of age pigs weaned at 4 weeks of age spent more time nosing other pigs and standing or sitting inactive, and less time exploring peat than pigs weaned at 6 weeks of age ($P<0.01$).

**Table 1** Influence of weaning at 4 or 6 weeks of age on the proportion of observation time spent in different behaviours during the first 4 weeks post weaning and between 6 and 10 weeks of age

| Behaviour | First 4 weeks post weaning | | | | 6 to 10 weeks of age | | | |
|---|---|---|---|---|---|---|---|---|
| | 4 weeks | 6 weeks | s.e.m. | P | 4 weeks | 6 weeks | s.e.m. | P |
| Nosing pig | 0.037 | 0.018 | 0.0048 | <0.05 | 0.045 | 0.018 | 0.0043 | <0.01 |
| Head thrusting | 0.005 | 0.003 | 0.0009 | <0.05 | 0.004 | 0.003 | 0.0005 | NS |
| Fighting | 0.005 | 0.001 | 0.0011 | <0.05 | 0.001 | 0.000 | 0.0007 | NS |
| Standing or sitting inactive | 0.126 | 0.056 | 0.0113 | <0.01 | 0.152 | 0.056 | 0.0120 | <0.001 |
| Exploring peat | 0.211 | 0.319 | 0.0231 | <0.01 | 0.206 | 0.319 | 0.0221 | <0.01 |

Pigs which were mixed at weaning rather than prior to weaning spent a greater proportion of time fighting during the first 4 weeks after weaning (mixed at weaning 0.006, mixed prior to weaning 0.001, s.em. 0.0011, $P<0.05$) and had a greater injury score to the shoulder area after weaning (mixed at weaning 1.88, mixed prior to weaning 0.81, s.em. 0.332, $P<0.05$). Pigs which were housed in groups of eighteen rather than groups of six spent a greater proportion of observation time nosing other pigs during the first 4 weeks after weaning (groups of eighteen: 0.035, groups of six 0.020, s.e.m. 0.0048, $P<0.05$) and between 6 and 10 weeks of age (groups of eighteen 0.039, groups of six 0.024, s.e.m. 0.0043, $P<0.05$). There were no other significant effects of group size or mixing prior to or after weaning on behaviour.

Pigs weaned at 4 and 6 weeks of age weighed 8.9 kg and 13.9 kg respectively (s.e.m. 0.36, $P<0.01$). There were no significant effects of weaning age, mixing strategy or group size on weights at 6 or 10 weeks of age. Overall mean weight at 6 weeks of age was 13.6 kg and at 10 weeks of age was 29.5 kg.

**Conclusions** The management practices investigated were important determinants of welfare but not productivity. Weaning age had the greatest effect of all treatment factors on levels of negative social behaviours post weaning. The fact that treatment effects on behaviour were shown not only during the post weaning period but also between 6 and 10 weeks of age suggest that behaviours learned in early life remain into the growing period.

## Alternatives to nose-ringing in outdoor sows: the provision of a special rooting area

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**Introduction** Nose-ringing is commonly used in outdoor pig production to prevent pasture damage. However, nose-ringing causes acute stress at the time of ringing and inhibition of normal rooting behaviour (Horrell *et al.* 2001). Therefore alternatives to nose-ringing need to be investigated. Previous studies have focused on dietary modification as a way of reducing rooting behaviour and pasture damage (e.g. Braund *et al*. 1998). Although these methods helped to reduce rooting behaviour they were ineffective at decreasing long-term pasture damage. This experiment examined the effectiveness of providing a special, enriched rooting area in an attempt to reduce pasture directed rooting behaviour and consequently reduce overall pasture damage.

**Materials and Methods** Four groups of 4 multiparous, pregnant sows were allocated to one of four treatments in a 4 by 4 Latin square design with 2 week periods. Treatment A had no rooting area. Treatments B-D had a rooting area consisting of a 15 x 2m strip of ploughed land supplemented with 200kg of spent mushroom compost. The compost was replenished between each period. Pigs on all treatments received the same ration of concentrate feed at 09.30h. Pigs on treatments A and B were fed on the ground on an area at the front of the paddock. Pigs on treatment C received half their daily ration on the ground and half buried in the rooting area. Pigs on Treatment D received their total daily concentrate ration buried in the rooting area. Behaviour observations were made on 3 days in each week over four, 1 hour sessions per day. The posture and behaviour of each pig was recorded at 5min intervals in each hour. The behaviours 'rooting the pasture', 'feeding from the ground' and 'using (feeding and rooting) the rooting area' are discussed here. Pasture damage was assessed weekly with a quadrat in a fixed sampling pattern across each paddock to estimate the percentage vegetation cover. Behaviour data, which were log 10 transformed to normalise, and pasture damage data were analysed using analysis of variance.

**Results** Fig. 1 shows the mean frequency of rooting the pasture, eating feed from the ground, and using the rooting area during the observation periods. There were no differences in the mean observed time spent rooting the pasture between treatments. However, pigs spent more of the observed time using the rooting area containing the total daily concentrate ration (Treatment D) than the rooting area containing half the daily feed ration (Treatment C). Pigs on Treatment C, in turn used their rooting area more frequently than pigs on Treatment B where the rooting area contained no food items (s.e.d. 0.032, $P<0.001$). There was a significant difference in the frequency that pigs were observed feeding from the ground. Pigs on treatments A and B spent more time feeding from the ground than pigs on Treatment C (s.e.d. 0.008, $P<0.001$); this is probably as only half the concentrate ration was fed on the ground in Treatment C. There was a significant difference in the percentage vegetation cover for each paddock in the last week (week 8) of the experiment ($P<0.001$) (see Fig. 2).

**Fig 1**. Mean percentage of observations that pigs were rooting the pasture, feeding from the ground and using the rooting area for each treatment

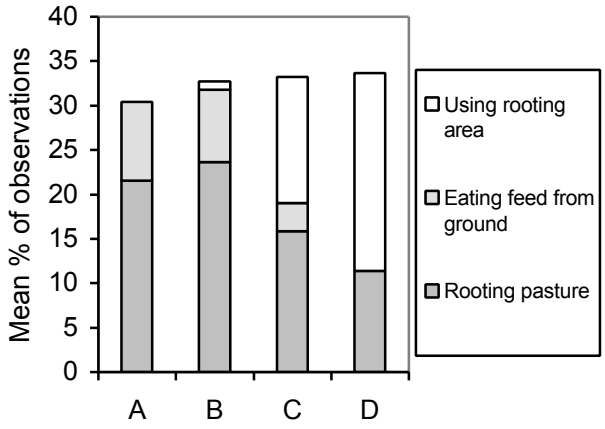
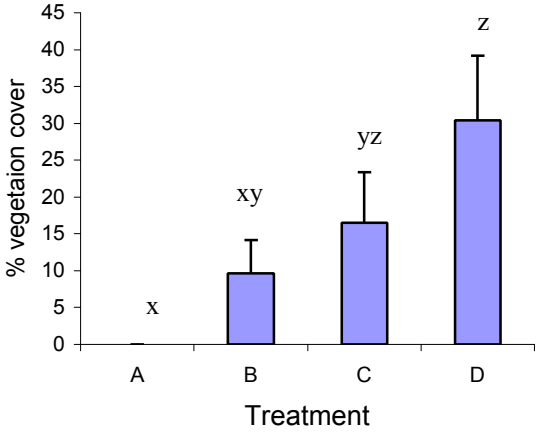



**Fig 2**. Percentage vegetation cover of each paddock in Week 8 (different letters indicate significant differences)




**Conclusions** The provision of a rooting area enriched with food items reduced the amount of pasture damage after an eight week period. This was reflected in the increased usage of the rooting areas when a proportion, or the entire daily feed ration was buried in them. However, the pasture damage incurred was still at a significant level; only 30% of the vegetation remained in Paddock D at the end of the experiment. Therefore, it is unlikely that the provision of a special rooting area would offer a complete alternative to nose-ringing in a commercial setting.

**Acknowledgements** This work was funded by the RSPCA.

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# The development of maintenance energy requirement and energetic efficiency for lactation from production data of lactating dairy cows

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**Introduction** The energy feeding systems currently adopted for dairy cows in Western Europe and North America were developed from calorimetric data published 30 years ago. However, the calorimetric measurements were usually undertaken with a small number of trained animals, housed for a short period in respiration chambers. The objective of the present study was to use production data to develop the metabolisable energy (ME) requirement for maintenance ($ME_m$) and the efficiency of ME use for lactation ($k_l$) for dairy cows.

**Material and methods** A total of 139 treatment mean data of lactating dairy cows (individual cows, n = 838), offered mixed diets of concentrates and silages of grass (n=33) and maize (n=5) *ad libitum*, were obtained from 12 long term feeding studies (at least eight weeks/period) across Northern and Southern Ireland. The dietary ME concentrations were either measured directly in calorimetry or estimated from the *in vivo* digestible organic matter in total dry matter (DM) (AFRC, 1993). The ME requirement for pregnancy was estimated for late pregnant cows and subtracted from total ME intake. The milk energy output ($E_l$) was calculated from milk composition and the live weight change (LWC) was estimated from linear regression between live weight and recording date. The energy balance ($E_g$) and then $E_{l(0)}$ ($E_l$ adjusted to zero $E_g$) were calculated using a range of data of energy contents per unit of LWC (19.0-26.8 MJ/kg, full live weight basis) and energetic efficiencies as suggested in a number of energy feeding systems (Van Es, 1978; INRA, 1989; AFRC, 1990 and 1993; SCA, 1990; NRC, 2001).

**Results** The grass and maize silages used covered a wide range in qualities and this, together with different forage proportions in diets (0.30-0.86), resulted in a range of ME concentrations in diets (10.3-12.6, mean 11.5 MJ/kg DM). Milk yield and LWC ranged respectively from 10.0 to 41.4 (mean 23.7) and from –0.405 to 0.825 (mean 0.234) kg/d. As the energy contents per unit of LWC and the energetic efficiencies suggested are different in the above feeding systems, mean calculated $E_{l(0)}$ values therefore varied marginally (from 0.707 to 0.721 MJ/kg$^{0.75}$). Consequently, the $ME_m$ and $k_l$ values, derived from the linear regression of ME intake against $E_{l(0)}$, ranged respectively from 0.59 to 0.62 (MJ/kg$^{0.75}$) and from 0.64 to 0.67 (Table 1). However, the parallel test on the linear relationship between ME intake and $E_{l(0)}$ revealed that the above difference in $E_{l(0)}$ between these systems had no significant effect on either intercept or slope, indicating that there were no significant differences in $ME_m$ or $k_l$ values derived from these systems. The mean $ME_m$ or $k_l$ across the feeding systems therefore was 0.60 (MJ/kg$^{0.75}$) or 0.65. The $ME_m$ and $k_l$ values derived from the above energy systems were then validated using the calorimetric data of lactating dairy cows published since 1976 (experiment mean, n=49). The validation indicated that predicted ME requirement was significantly related to actual ME intake for each system ($P<0.001$, $R^2$ = 0.93-0.94). The prediction bias only accounted for 0.003-0.014 of actual ME intake. The prediction error was mainly derived from random (0.93 to 0.98 of total mean-square-prediction-error).

**Table 1.** Relationships between ME intake (x) and $E_{l(0)}$ (y) (calculated using energy content per unit of LWC and energetic efficiencies recommended in different energy feeding system, the values in brackets are s.e. data)

| System | Coefficient ($k_l$) | Intercept | $R^2$ | $ME_m$ (MJ/kg$^{0.75}$) |
|---|---|---|---|---|
| AFRC (1990) | 0.663 (0.0290) | -0.405 (0.0499) | 0.81 | 0.611 |
| AFRC (1993) | 0.640 (0.0278) | -0.376 (0.0477) | 0.81 | 0.588 |
| SCA (1990) | 0.654 (0.0285) | -0.396 (0.0490) | 0.81 | 0.604 |
| Van Es (1978) | 0.645 (0.0280) | -0.382 (0.0481) | 0.81 | 0.592 |
| INRA (1989) | 0.674 (0.0297) | -0.419 (0.0510) | 0.80 | 0.622 |
| NRC (2001) | 0.650 (0.0282) | -0.390 (0.085) | 0.81 | 0.600 |
| Mean | 0.654 | | | 0.603 |

**Conclusion** The $ME_m$ and $k_l$ values derived from the present production data are within the ranges reported recently from the calorimetric data of lactating dairy cows.

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# The effect of cow genotype on energy partitioning between milk and body tissue

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**Introduction** In an extensive review of energy metabolism of dairy cows, Agnew and Yan (2000) concluded that high genetic merit cows are capable of partitioning more energy into milk and less into body tissue when compared to low genetic merit cows. The objective of the present study was to use production data for a complete lactation to evaluate the effect of cow genotype on energy partitioning between milk and body tissue.

**Material and methods** Two breeds of first lactation dairy cattle (Holstein Friesian (HF) *v.* Norwegian cattle (NC)) (n=32 for each) were offered a mixed diet of grass silage and concentrates from calving to 44 weeks of lactation. The animals of HF ($PIN_{(00)}$ £44) and NC (total merit index of 10.1) were representative of the top 1% and 5% of the HF in UK and NC in Norway respectively. The heifers were blocked into pairs within breed and allocated at random to diets containing either a high or low proportion of concentrates. The concentrate proportions in diets (high *v.* low) for 1-100, 101-200 and 201-300 days of lactation were 0.60 *v*. 0.30, 0.50 *v.* 0.20 and 0.40 *v*. 0.10, respectively. 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. All cows were offered the diets *ad libitum* and in addition received the equivalent of 160 g/d of a mineral vitamin pre-mix. DM intake, milk production and live weight of the cattle were recorded throughout the present study. The metabolisable energy (ME) concentration in diets was measured in calorimetric chambers using eight cattle of each breed at days 80, 160 and 240 of lactation and the mean dietary ME concentration for each breed was directly applied to production data for all animals within breed. Milk energy output ($E_l$) was calculated using actual fat, protein and lactose concentrations in milk. Energy balance ($E_g$) was estimated as the difference between ME intake (MEI) and ME used for maintenance and lactation and then multiplied by the efficiency of ME use for live weight gain (AFRC, 1990).

**Results** The results of mean data of individual cows across the complete lactation are presented in Table 1. With the low proportion of concentrates, there were no significant differences between two breeds in terms of MEI, $E_g$ and $E_l$/MEI or $E_g$/MEI, although HF had a higher $E_l$ ($P<0.01$) and $E_{l(0)}$/MEI ($P<0.05$) than NC ($E_{l(0)}$ is $E_l$ adjusted to zero $E_g$). However, with the high proportion of concentrates, all variables, except for $E_g$ and $E_g$/MEI which were similar between two breeds, were significantly higher with HF than NC ($P<0.01$ or less). The findings indicate that the potential for milk production and the high $E_l$/MEI with the HF cows increase as the proportion of concentrates increases. The mean data of $E_l$/MEI and $E_g$/MEI of each lactation week across treatment of cows are presented in Figure 1. With the high proportion of concentrates, $E_l$/MEI was considerably higher with HF than NC at beginning of lactation and then the difference was gradually reduced with lactation until 25 weeks. Contrarily, NC has a higher $E_g$/MEI than HF and the trend of difference was similar to $E_l$/MEI, but at the late stage of lactation HF partitioned more ME into tissue than NC for compensating high loss of weight at the early stage of lactation and growth. With the low proportion of concentrates, there were similar patterns in these two variables between the two breeds, but the differences disappeared after a few weeks of lactation and then these two variables were very similar between the two breeds.

**Table 1**. Effect of breeds and levels of concentrates

| | High conc. level | | Low conc. level | | |
|---|---|---|---|---|---|
| | HF | NC | HF | NC | s.e.d. |
| MEI (MJ/d) | 196$^{c}$ | 177$^{b}$ | 145$^{a}$ | 138$^{a}$ | 4.7 |
| $E_l$ (MJ/d) | 76$^{d}$ | 63$^{c}$ | 53$^{b}$ | 47$^{a}$ | 1.8 |
| $E_g$ (MJ/d) | 20$^{b}$ | 19$^{b}$ | 9$^{a}$ | 10$^{a}$ | 2.2 |
| $E_l$/MEI | 0.387$^{b}$ | 0.357$^{a}$ | 0.362$^{a}$ | 0.343$^{a}$ | 0.0095 |
| $E_g$/MEI | 0.100$^{b}$ | 0.105$^{b}$ | 0.063$^{a}$ | 0.071$^{a}$ | 0.0116 |
| $E_{l(0)}$/MEI | 0.487$^{d}$ | 0.463$^{c}$ | 0.425$^{b}$ | 0.414$^{a}$ | 0.0049 |

$^{a,b,c,d}$ Superscript means with the same letter in the same row are not significantly different ($P>0.05$)

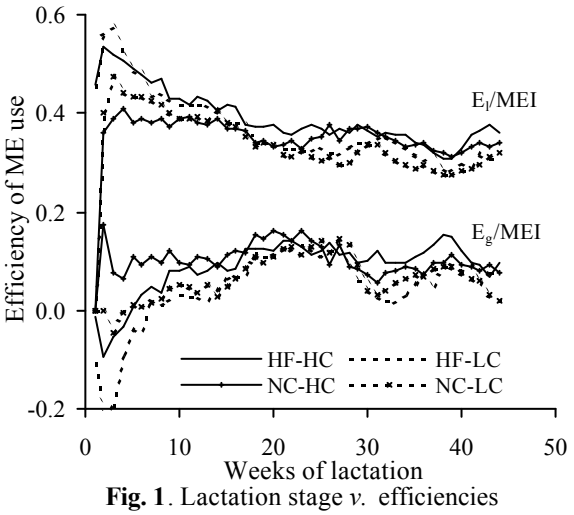


**Fig. 1**. Lactation stage *v.* efficiencies

**Conclusion**. HF cows have the ability to partition more energy into milk and less into body tissue than NC cows, but this potential is more likely to be achieved with a high plane of nutrition and with cattle at early-mid stage of lactation.

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# An investigation of the efficiency of nutrient utilization for milk production by Holstein and Norwegian breeds of dairy cattle

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**Introduction** Holstein-Friesian cattle are the predominant breed of dairy cattle in Northern Ireland. Breeding programmes for the Holstein Friesian have 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 via a multi-trait selection procedure and there is evidence that problems associated with disease and fertility have tended to decrease in recent years. It is important, however, to investigate possible differences in efficiency of food use and partitioning of nutrients between the two breeds which may offset the potential advantages of improvements in secondary characteristics. Consequently, the objectives of this experiment were firstly to investigate the effects of breed type on the efficiency of utilization and partitioning of nutrients for milk production and secondly to investigate if there are differences in the energy requirement for maintenance and the efficiency of utilization of ME for lactation ($k_l$) between the two breeds.

**Materials and methods** Sixteen first lactation dairy cows were used in this 2 (breed) * 2 (plane of nutrition) factorial design study. Eight were Holstein Friesian (PIN $_{(00)}$ £44 and 8 were Norwegian dairy cattle (total merit index of 10.1). Each breed of dairy cattle 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. Energy metabolism studies were undertaken on each animal at day 80, 160 and 240 of lactation. Each cow was placed in open-circuit indirect respiration calorimeters for 72 hours, with measurements of gaseous exchange during the final 48-hour period in the chambers being used to calculate heat production. On completion of each three-day period in the respiration chambers, animals were tied in individual standings in a cowshed and a six-day ration digestibility study undertaken. Data were analyzed by ANOVA to examine effects of breed, plane of nutrition and stage of lactation and their interactions. The 24 data for each breed were also used to determine if there were differences in the energy requirement for maintenance ($ME_m$) or the efficiency of use of ME for lactation ($k_l$) between the two breeds. Linear regression of milk energy output (adjusted to zero energy balance) (AFRC, 1990) against ME intake was undertaken.

**Results** No significant interactions (Table 1) were observed between breeds and plane of nutrition with the exception of milk energy output, which was significantly higher for the Holstein dairy cattle on both planes of nutrition. The milk energy output of the Norwegian dairy cattle offered the high plane of nutrition was also significantly higher than for the Norwegian dairy cattle offered the low plane of nutrition. Whilst there were no significant differences in the partitioning of ME intake into milk energy output between the breeds or planes of nutrition, the Holstein dairy cattle on the high plane of nutrition tended to partition more ME intake towards milk than any of the other treatments. On the two high planes of nutrition both breeds retained energy, whilst on the low planes of nutrition there was a small loss of body tissue. These differences were not significant. The $ME_m$ and $k_l$ derived from the linear regression analysis were both higher for the Holstein cows than the Norwegian cows. However these differences were not significantly different.

Table 1 Energy utilization of Holstein and Norwegian dairy cattle

| | Holstein | | Norwegian | | | |
|---|---|---|---|---|---|---|
| | High | Low | High | Low | SEM | Sig |
| ME intake (MJ/d) | 210 | 157 | 192 | 144 | 6.37 | NS |
| Milk energy output (MJ/d) | 75.5 | 52.6 | 62.8 | 46.4 | 1.32 | * |
| Retained energy (MJ/d) | 9.9 | -0.6 | 4.2 | -3.0 | 3.86 | NS |
| Heat production (MJ/d) | 125 | 105 | 125 | 101 | 4.31 | NS |
| ME conc (MJ/kg DM) | 11.9 | 11.4 | 11.8 | 11.4 | 0.08 | NS |
| $k_l$ (AFRC 1990) | 0.55 | 0.51 | 0.50 | 0.51 | 0.016 | NS |

**Conclusions** There are no significant differences in the energy utilisation of Holstein or Norwegian dairy cattle.

**Reference**

AFRC. 1990. Technical Committee on Responses to Nutrients, Report No 5, Nutritive Requirements of Ruminant Animals: Energy. *Nutrition Abstracts and Reviews (Series B)* **60**:729-804.

# Energy balance profiles for the first three lactations of dairy cows estimated using random regression

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**Introduction** The replacement of lost body tissue in modern dairy cows usually takes place later in the same lactation, once milk output begins to decline. Individual cows may not regain all lost body energy in the first lactation leading to a greater deficit to be replenished in the second. This results in carry-over effects from one lactation to the next, on both yield and non-yield traits. The use of random regressions and Fourier series allows modelling of cyclical changes in a trait over seasons (Meyer, 2000) and could be used to model multiple lactation energy balance changes in dairy cows. Parameters of these cyclical functions might then be analysed for relationships with traits of economic importance such as health, fertility and survival. The objectives of this study were 1) to model phenotypic daily milk yield, fresh feed intake, liveweight and condition score using random regression techniques, 2) to combine daily estimates obtained in objective 1) into an overall energy balance for each day of the first three successive lactations, 3) to compare energy balance curves over three lactations and 4) to investigate the feasibility of modelling energy balance in the first three lactations using harmonic analysis.

**Materials and Methods** Data was extracted for animals calving at the Langhill Dairy Cattle Research Centre since 1990 and included records of milk production and composition, liveweight (LWT), condition score (CS) and feed intake (FI). Daily animal solutions were predicted using random regression analysis for feed intake, milk yield, liveweight and condition score recorded on 189 cows that had three successive lactations. Energy balance for days 1 to 305 of each of the three lactations was calculated both from daily measures of feed intake and milk output and from weekly measures of liveweight and condition score. Energy balance over 3 lactations was modelled using sinusoidal functions which were associated with individual cows and allowed to vary between cows. The parameters of these curves are potentially useful since they have a biological interpretation. The phase relates to the period from calving to return to positive energy balance and the amplitude relates to the degree of body energy loss.

**Results** Cows returned to positive energy balance at days 72, 75 and 95 in lactations 1, 2 and 3 respectively based on energy balance calculated from feed intake and milk output records (EB1), and at days 77, 83 and 73 based on energy balance calculated from body energy state changes (EB2). Differences in return to positive energy balance between EB1 and EB2 were non-significant in lactations 1 and 2 but significant in lactation 3. Correlations between energy balance at the same time in successive lactations ranged from 0.01 to 0.66 depending on the method of calculation and the stage of lactation.

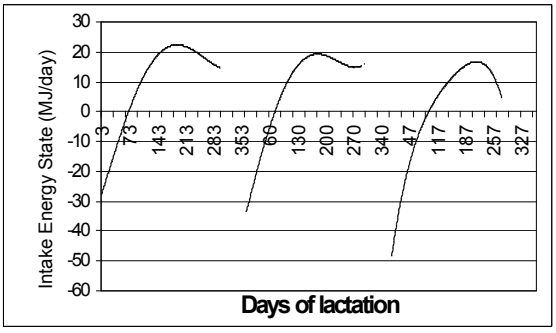


Figure 1. Three lactation energy balance using EB1.

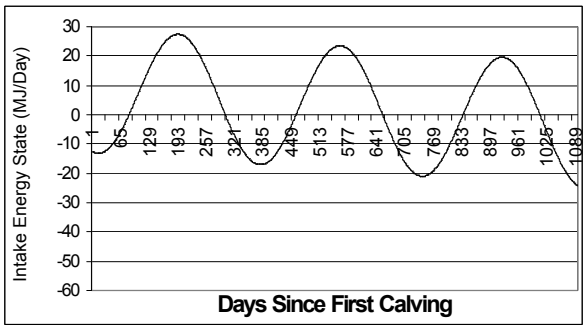


Figure 2. Three lactation energy balance using EB1 with sinusoidal fitting

The sinusoidal functions fitted to the curve removed a significant proportion of the variation but accounted for only 45% and 40% of the variation in EB1 and EB2 respectively.

**Conclusions** The relationship between energy balance in the first 3 lactations is likely to be more complex than a simple linear function but the profile of energy balance over the first 3 lactations may be a useful selection criteria in a multi-trait index. Energy balance profile over lactations 1 to 3 can be modelled with moderate accuracy using sinusoidal functions and warrants further investigation.

**Acknowledgements** We are grateful to Professor W G Hill who provided useful guidance and comments on the text and Ian White is acknowledged for his help with the analysis. SAC receives financial support from the Scottish Executive Rural Affairs Department.

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# The effect of stocking rate on the performance of two breeds of dairy cattle at pasture

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**Introduction** In Northern Ireland, grazed grass is the main diet offered to dairy cattle throughout the summer months. Whilst the Holstein-Friesian (HF) dairy cow is extremely efficient at converting grass into milk, there is evidence that the incidence of infertility in this breed is increasing (Mayne *et al*. 2001). This may reflect the fact that the breeding goals for the HF breed have been based primarily on selection for milk production with little emphasis for other traits such as fertility or health. In contrast, Norwegian dairy cattle (NC) have been selected using a multi trait index, with less emphasis on milk production and inclusion of other fertility and health traits. The overall objective of the present study was to evaluate the effect of varying stocking rate at pasture on performance of these two contrasting breeds.

**Materials and Methods** Twenty-four HF (PIN $_{(00)}$ £41) and twenty-four NC (TMI=10.2) were involved in the study. These animals were representative of the top 1% and top 5% of cattle in the Netherlands and Norway respectively. Mean calving dates of the HF and NC were 18 February and 2 March respectively. Within each breed, animals were grazed at either a low or a high stocking rate. Prior to turnout, all animals were offered a total mixed ration *ad libitum*, containing grass silage and 9kg of concentrate per head per day.

Prior to the experiment, animals were paired within breeds on the basis of milk yield, liveweight, condition score, calving date, parity and genetic merit and allocated to either a low or high stocking rate treatment. Animals on the low stocking rate were allocated 20 % more area than recommended guidelines while the animals on the high stocking rate were allocated 20% less area than recommended guidelines. Within treatments, animals grazed in a 1-day paddock system, with the animals entering a new paddock after the evening milking. The experimental period lasted from the 9 May until the 19 September. Throughout the experimental period, animals were offered 3kg of concentrates daily. Herbage mass was determined from herbage clips taken twice weekly pre- and post-grazing throughout the grazing season and grass heights were assessed daily using a rising plate meter (Ashgrove, New Zealand).

**Results** The average pre grazing sward heights were 13.4 and 12.4 cm for the low and high stocking rates respectively, with residual sward heights of 7.4 and 5.7 cm for the low and high stocking rates respectively. Treatment effects on animal performance are given in Table 1. Daily milk yields were significantly ($P<0.01$) lower for the NC compared to the HF (22.2 vs 24.4kg) with a mean milk response of 2.0 and 1.8 kg/d to the decrease in stocking rate for the NC and HF respectively. There were no significant ($P<0.05$) differences in milk composition between either breeds or treatments. Animals on the low stocking rate had greater live-weight gain over the experimental period compared to those on the high stocking rate (29.5 vs 5.0 kg). NC had a significantly ($P<0.001$) higher condition score than HF cattle at both the start and the end of the experiment on both stocking rate treatments.

**Table 1** Effects of stocking rate and breed on animal performance

| | Low Stocking Rate | | High Stocking Rate | | | Significance | | |
|---|---|---|---|---|---|---|---|---|
| | HF | NC | HF | NC | Se | Breed | Treat | Interaction |
| Milk yield (kg/d) | 25.3 | 23.2 | 23.5 | 21.2 | 1.12 | ** | * | NS |
| Milk fat (g/kg) | 40.3 | 40.2 | 41.0 | 40.3 | 1.50 | NS | NS | NS |
| Milk protein (g/kg) | 34.2 | 34.0 | 34.6 | 33.6 | 1.03 | NS | NS | NS |
| Initial liveweight (kg) | 512 | 483 | 511 | 490 | 14.3 | NS | NS | NS |
| Final liveweight (kg) | 545 | 509 | 514 | 497 | 15.2 | NS | NS | NS |
| Initial condition score | 2.42 | 2.88 | 2.38 | 2.80 | 0.091 | *** | NS | NS |
| Final condition score | 2.23 | 2.68 | 2.27 | 2.59 | 0.079 | *** | NS | NS |

**Conclusions** These results indicate that HF animals, selected primarily for milk production, had a higher milk production than NC, but there were no breed differences in milk response with a decrease in stocking density. There was no significant difference in live-weight or condition score change between breeds, even though the HF animals produced more milk. This may be due to greater grass intakes with the HF breed.

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# Estimation of genetic variation in plasma leptin concentrations in pre-pubertal heifers.

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**Introduction** Leptin is involved in the regulation of food intake, energy expenditure and whole body energy balance. In livestock species these processes are important for optimisation of growth, reproduction, lactation and overall health and well-being. Condition score is a useful indicator of energy balance and is highly correlated (-0.84) genetically to commencement of luteal activity (CLA), an endocrine measurement of fertility in dairy cattle (Royal *et al*., submitted). Circulating leptin levels drop *post-partum* reflecting changes in energy balance and fat mobilisation. The interval to first ovulation *post-partum* is phenotypically correlated (0.83) to the interval from parturition to the leptin nadir (Kadokawa *et al*. 2000), suggesting that a delay in the recovery of leptin secretion postpartum increases the delay to first ovulation. The heritability of circulating leptin concentrations in humans and pigs is high (0.39-0.61; Cameron *et al*., 2000; Rotimi *et al*., 1997). If this were also the case in cattle, and postpartum changes were related to concentrations at an early age, then it would be of immense value to the genetic improvement of traits associated with fertility.

**Materials and Methods** A database constructed for previous work consisting of 158 paternal half-sib families varying between 1 and 23 daughters/sire was used. All animals (Holstein-Friesian heifers; n=560) were selected from seven commercial farms using sire and age (120-140 days) as selection criteria. Heparinised blood samples were collected from the jugular vein. Concentrations of leptin were measured using a recently developed specific RIA (Blache *et al*., 2000), with a mean sensitivity of 0.2 $ng.ml^{-1}$ and an intra assay coefficient of variation of 9.8%. The data were analysed using ASREML. The magnitude of variance components, including the genetic variance, and mean values for the fixed effects were estimated using a univariate model. Functions of the variance components of particular interest were the phenotypic variance, the additive genetic variance and the heritability. A bivariate model was then used to estimate genetic, phenotypic and environmental correlations with live weight (kg) at the same age.

**Results** Mean plasma leptin concentration was 0.86 ng/ml (s.e. 0.02) with a range of 0.2 – 2.74 ng/ml. The distribution was skewed (0.91) so all further analyses used the natural log transformation. Average weight of the heifers was 120.13kg (se; 0.84).

**Table 1** Estimate of variance components and heritability estimates after fitting fixed effects into the model

| Trait | $\sigma^2_S$ (s.e.) | $\sigma^2_e$ (s.e.) | $\sigma^2_A$ | $\sigma^2_P$ | $h^2$ (s.e.) |
|---|---|---|---|---|---|
| Leptin (ng/ml) | 0.00 | 0.1893 (0.0130) | 0.00 | 0.1893 | 0.00 |
| Weight (kg) | 0.067 (0.067) | 1.4012 (0.1051) | 0.268 | 1.4682 | 0.18 (0.18) |

$\sigma^2_S$, sire variance; $\sigma^2_e$, residual variance; $\sigma^2_A$, additive genetic variance, $\sigma^2_P$, phenotypic variance.

Following bivariate analysis phenotypic and environmental correlations between leptin concentration and weight were 0.14 (0.05) and 0.14 (0.05) respectively. Therefore, leptin concentrations increased with increasing live weight.

**Conclusion** These analyses provide evidence to show that additive genetic variation is not responsible for variation in plasma leptin concentration in pre-pubertal heifers. Therefore, simple measures of endogenous leptin concentrations are unlikely to be of value for selection programmes as a genetic predictor of merit as was the case in pigs (Cameron *et al*., 2000). However, since a high estimate of heritability was obtained in pigs at later stages of maturity (in relation to fat deposition) it could be hypothesized that genetic variation in dairy heifers may increase following expression of different genes in later life. Further, it may be that it is only after metabolic challenge that genetic variation can be observed at this age, analogous to the results of Woolliams and Lovendahl (1991).

**Acknowledgements** The authors thank DEFRA for funding this work and the technical help of Mr Marcus Mitchell and Mrs Morag Hunter.

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# Association of leptin polymorphisms with milk yield, dry matter intake, energy balance, luteal activity and serum leptin levels during lactation in HF dairy cows

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**Introduction** Since evidence is present that genetic correlations between start of luteal activity and energy balance, milk yield and live weight exist (Veerkamp et al., 2000), it could be hypothesised that polymorphisms at the leptin gene locus might play a role. The first objective of this study was to associate plasma leptin levels during late pregnancy with genetic differences in the leptin gene. The second objective was to relate these polymorphisms with variations in energy balance, milk production, dry matter intake and fertility.

**Materials and Methods** Animals were typed for two restriction fragment length polymorphisms (RFLP) (Pomp et al., 1997; Haegeman et al., 2000) and the BM1500 microsatellite (Fitzsimmons et al., 1998) at the bovine leptin gene. From late pregnancy until 80 days after calving blood samples were taken every two weeks for 323 cows. Leptin concentrations in these samples (Delavaud et al., 2000) were smoothed using the spline function in ASREML. Contrasts between genotypes were estimated including the spline for lactation stage in the model, effects for sample date, genetic group and a quadratic polynomial for calving-age. Sire and animal were fitted as random effects. For 613 Holstein Friesian (HF) heifers dry matter intake (DMI), milk yield (MY), energy balance (EB) and corpus luteum activity (CLA) where measured during the first 80 days of lactation. Contrasts between genotypes for the measured traits were estimated. Fixed effects were year-season (n=20), genetic group (n=2), a quadratic polynomial for age at calving and a random effect for the additive genetic relationship matrix.

**Results** Plasma leptin levels before parturition and during lactation are shown in Figure 1. No difference in leptin concentrations between the RFLP1 genotypes were observed (Table 1) but the RFLP2-BB genotype and the BM1500-B-allele had higher ($P<0.10$ and $P<0.05$) leptin levels than the other genotypes. The RFLP1-AB genotype was found to be associated with a higher MY ($\Delta$= 1.32 kg/d, P = 0.027) in the first 80 days of lactation compared to RFLP1-AA (Table 2).

**Table 1.** Average plasma leptin (±s.e.) (ng/ml) for each genotype (n=323).

| | **AA** | **AB** | **BB** | **AC** | **BC** | **CC** |
|---|---|---|---|---|---|---|
| **BM1500** | 3.83 ± 0.13 | 4.02 ± 0.10 | 4.17 ± 0.11 | 3.83 ± 0.14 | 4.17 ± 0.15 | 3.93 ± 0.20 |
| **RFLP2** | 3.95 ± 0.09 | 4.05 ± 0.10 | 4.30 ± 0.17 | | | |
| **RFLP1** | 3.99 ± 0.08 | 4.02 ± 0.13 | *not present* | | | |

**Table 2.** P-values for each polymorphism (n=613).

| | **RFLP1** | **RFLP2** | **BM1500** |
|---|---|---|---|
| **MY (kg/d)** | 0.027 | 0.970 | 0.963 |
| **DMI (kg/d)** | 0.087 | 0.445 | 0.876 |
| **EB (MJ/d)** | 0.806 | 0.262 | 0.930 |
| **CLA (d)** | 0.823 | 0.281 | 0.869 |

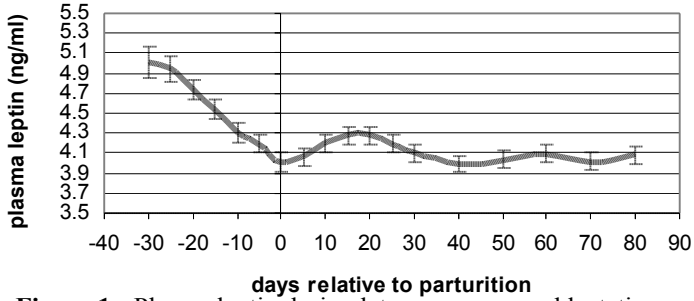


**Figure 1:** Plasma leptin during late pregnancy and lactation

**Conclusion** Whereas RFLP1 is associated with milk yield and tend to be associated with dry matter intake, it is not associated with mean leptin levels. In contrast, RFLP2 and BM1500 are not associated with any of the measured traits but are associated with leptin levels. A more detailed study of relationships between leptin levels and the studied traits is necessary to improve our understanding of the associations found and, more generally, the role of leptin in dairy cows.

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# Lactation induces hypoleptinaemia and activates orexigenic hypothalamic pathways in sheep

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**Introduction** The initial period of lactation in domestic ruminants is usually characterised by both hyperphagia and negative energy balance (Barber *et al,* 1997). The factors regulating the hyperphagia of lactation are not well understood. Leptin is a peptide hormone which is secreted by adipocytes, and which acts on a number of neuropeptides and receptors in the hypothalamus to regulate appetite and energy balance (Spiegelman and Flier, 2001). The aim of this study was to investigate the role of leptin in the hyperphagia of lactation in sheep.

**Material and methods** Multiparous Finn x Dorset crossbred ewes were used. Eight were non-pregnant non-lactating controls and 16 were suckling either 2 or 3 lambs. Ewes were fed *ad. lib.* with "hay saver" pellets that contained sodium hydroxide treated straw and dried grass. In addition ewes received a daily fixed amount of concentrate (Lactaters:1.2kg/d ; Controls:0.5kg/d). Lactating ewes were killed on day 6 and day 18 of lactation. Food intake was measured daily in lactating ewes and one week prior to killing in controls. Three blood samples were collected at 2h intervals on the day before killing and one the following morning immediately before killing. Plasma samples were analysed for leptin and free fatty acid (FFA) concentrations. Adipose tissue samples and brains were collected after killing. Adipose tissue was analysed for lipogenesis, adipocyte cell volume and leptin mRNA concentration. Gene expression (mRNA) of leptin receptor (OB-Rb), proopiomelanocortin, (POMC), agouti-related peptide (AGRP), melanocortin receptor 3 (MC3-R), neuropeptide Y (NPY) and cocaine-amphetamine-regulated transcript (CART), hypothalamic neuropeptide and receptor systems involved in appetite regulation, was quantified using *in situ* hybridisation. Results were analysed by ANOVA with physiological state as factor.

**Results** Food intake increased during the first 2 weeks of lactation and was significantly higher in lactating compared to non-lactating ewes. FFA levels was increased on both day 6 and 18 of lactation. Adipose tissue lipogenesis was significantly reduced in lactating ewes as was the volume of the adipocytes, indicating a loss of adiposity in lactating ewes. Plasma leptin concentration was 4-5 times higher in non- compared to lactating animals as shown in figure 1. OB-Rb gene expression increased during lactation in the hypothalamic arcuate nucleus (ARC) and the ventromedial nucleus (VMH). POMC gene expression was decreased, while AGRP was increased in the ARC by lactation. MC3-R expression was similar in lactating and non-lactating animals in both ARC and VMH. Lactation increased gene expression of NPY significantly in ARC and the dorsomedial nucleus (DMH). However expression in the DMH was only significantly higher at day 18 of lactation. CART mRNA expression decreased during lactation in both ARC and VMH.

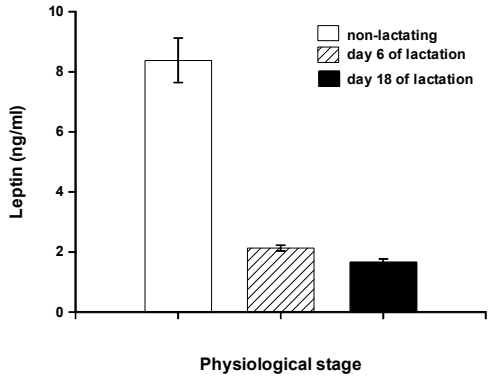


**Figure 1.** Plasma leptin concentrations (ng/ml) during lactation in sheep.

**Conclusions** Lactation induced a state of negative energy balance and hypoleptinaemia despite food being available *ad. libitum*. The hypoleptinaemia may be responsible for activating the orexigenic NPY pathway, and decreasing activity of the anorexic melanocortin and CART pathways resulting in hyperphagia during early lactation in sheep. Despite this increased orexigenic drive, the sheep remained in negative energy balance. The reason for sheep continuing in negative energy is still to be resolved.

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**Acknowledgements**

We thank the Scottish Executive Environmental and Rural Affairs Department for financial support

# Long-term effect of food intake on adipose tissue and leptin secretion during long days in Soay rams

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**Introduction** Circulating leptin, the obese gene product secreted by adipocytes, is sensitive to short-term (meals, starvation) and long-term (spontaneous food intake, food restriction) changes in energy balance in sheep (Marie *et al* 2001). The present study aimed to explore relationships between adiposity, adipose tissue from different sites and blood leptin concentrations in rams kept in long days when adiposity is normally high.

**Materials and methods** Adult Soay rams were individually housed in artificial light-controlled rooms, allowing contact by smell, touch and vision. After 16 weeks in short days (8 h light:16 h darkness), they were exposed for 16 weeks to long days (16 h light:8 h darkness), during which half (AL, n=6) were fed *ad libitum* a complete diet (50% chopped hay, 30% rolled barley, 9% white fishmeal), and half (R, n=6) were restricted to 90% liveweight maintenance. Groups were balanced regarding short days animal weight and food intake. Body condition score (BCS) and liveweight were measured fortnightly. Insulin, glucose and non-esterified fatty acids (NEFA) concentrations were measured in plasma samples obtained hourly over 24 h one week before slaughter. On the day of slaughter, blood was sampled for leptin assay (Marie *et al* 2001), and adipose tissue was collected from the following sites: subcutaneous (C, obtained on one side, between the hind rib and hip), inguinal (I), omental (O) and peri-renal (R), weighed and osmium tetroxyde-fixed (Etherton *et al* 1977). Mean cell diameter and surface were measured on 200 cells from each site in each animal, using an image analyser (Image ProPlus). Statistical analyses (comparisons between groups, and between tissues within groups [using animals as blocks] by ANOVA, and correlations) were performed using Genstat5. All experimental procedures were licensed under the UK Animals (Scientific Procedures) Act, 1986, and received approval by the Rowett Research Institute's Ethical Review Committee.

**Results** Voluntary food intake markedly increased in AL rams in long days (from 742±43 in short days to 1517±86 g DM/day), resulting in increased liveweight and BCS, while food restriction in R rams was associated with weight loss and decreased BCS (Table 1). Fat depots were 10 fold heavier (g) in AL than R rams : C, 116±38$^{b}$ v 10±3$^{c}$ ; I, 53±15$^{b}$ v 6±1$^{c}$; O, 678±138$^{a}$ v 57±25$^{b}$; R, 569±105$^{a}$ v 48±14$^{b}$. Adipocyte diameter (µm) was more than doubled in AL v R rams: C, 86.8±10.1$^{c}$ v 45.3±10.1$^{d}$; I, 118.1±8.3$^{b}$ v 49.5±8.3$^{d}$; O, 149.7±6.4$^{a}$ v 59.7±10.3$^{d}$; R, 131.7±3.2$^{ab}$ v 55.9±10.7$^{d}$. Adipocyte area and leptin secretion were also significantly higher in AL than R sheep (Table 1).

**Table 1** Changes in liveweight and BCS during 16 weeks of feeding regimen, and final adipocyte area and plasma leptin concentration. Mean values (s.e.m.) with different superscripts in a column or between tissues are significantly different (P<0.05).

| Group | N | Food intake (g DM/day) | Weight change (kg) | BCS change | Adipocyte area (µm$^2$) C | I | O | R | Leptin (ng/ml) |
|---|---|---|---|---|---|---|---|---|---|
| AL | 6 | 1517 $^{a}$ (86) | + 10.5 $^{a}$ (1.9) | + 0.39 $^{a}$ (0.06) | 7195 $^{c}$ (1668) | 11944 $^{b}$ (1778) | 19008 $^{a}$ (1473) | 14608 $^{b}$ (700) | 7.87 $^{a}$ (1.78) |
| R | 6 | 660 $^{b}$ (0) | - 5.2 $^{b}$ (1.4) | -0.75 $^{b}$ (0.06) | 2809 $^{d}$ (1114) | 2442 $^{d}$ (571) | 3475 $^{d}$ (968) | 3166 $^{d}$ (908) | 1.78 $^{b}$ (0.13) |

Plasma leptin was positively and highly significantly (P<0.01) correlated to liveweight change (r=0.815) and BCS change (r=0.734), to plasma insulin (r=0.916) and to all fat tissues and adipocytes measurements, but not to plasma glucose (r=0.535, P=0.07). Leptin was negatively correlated with NEFA (r=-0.747). Correlation coefficients between adipocyte area and BCS or weight change were lower for subcutaneous tissue (0.666 or 0.599, respectively) than for inguinal (0.863 or 0.879), omental (0.908 or 0.923) or peri-renal (0.910 or 0.929) tissues. In fact, subcutaneous cell size was only increased in rams with the greatest weight gain.

**Conclusions** The dramatic decrease in body condition and adipocyte size induced by the moderate reduction in food intake in long days was indicative of an enhanced mobilisation of body reserves, which could be the sign of high turn-over of energy metabolism in this period. The results of the present study show that circulating leptin concentration is a good indicator of body fat reserves in the absence of short-term variations in food intake. The greater difference between the groups seen in the size of adipocytes from internal (O,R) compared with subcutaneous (C) sites, and the closer correlation between adipocyte size and BCS or weight change for the former sites suggest that fat deposition and lipogenesis occur preferentially in internal as opposed to subcutaneous adipose tissue in rams.

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## Recombinant leptin mutants and leptin binding proteins aimed to block leptin action in vivo

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**Introduction** Obese protein (OB) also known as leptin serves as a protein signal secreted from adipose tissue and acts on a central nervous system that regulate ingestive behavior and energy balance (Campfield, 2000). The sequence of various leptins from 10 mammalian species was compiled and the 3D structure of human leptin mutant W100E was elucidated (Zhang et al., 1997). We have prepared recombinant leptins of sheep, chicken, cow and pig. Mammalian and chicken leptins are respectively 146 and 145 amino acid containing proteins found in circulation. Leptin in blood is found in both free and bound form; the main binding protein is the extracellular domain (ECD) of leptin receptor (Liu et al., 1997). One of the established functions of leptin, is its attenuating effect on the expression of NPY and other neuropeptides in hypothalamus that subsequently leads to decreased food intake (Campfield, 2000). Therefore it seems logical that blocking leptin receptors that are responsible for transferring it through the blood-brain barrier or for its action in hypothalamus will lead to increase in food intake. Leptin receptor belongs to cytokine receptor superfamily. Its ECD consists of ~ 800 amino acids but it was suggested that only the cytokine homology subdomain II (CHD) consisting of ~ 200 amino acids is responsible for binding (Fong et al., 1997). The objective of the present work is to prepare recombinant proteins aimed to block leptin action. We suggest two approaches (a) preparation of leptin antagonists capable of binding but not homodimerizing leptin receptors and (b) subcloning and preparing CHD II of leptin receptor responsible for binding of the hormone.

**Methods** Three basic methodologies were used for preparation of recombinant proteins or their mutants: (a) Methodology based on PCR technology. PCR oligonucleotides (primers) containing the DNA for isolating the desired genes or to produce mutations and appropriate restriction enzyme sites were used along with pMON3922 vector or leptin or leptin receptor DNA templates; (b) Methodology based on a modification of respective expression plasmids using the Stratagene Quickchange$^{TM}$ mutagenesis kit using two complementary primers; and (c) Methodology based on error prone mutagenesis of ovine leptin presented on phage and screening by phage display methodology. To prepare the recombinant proteins, plasmids encoding the specific protein were used to transform *E. coli* MON105 cells for expression as insoluble inclusion bodies (aggregates). The recombinant proteins were then prepared by solubilizing the inclusion bodies in 4.5 M urea at high pH at presence of reducing agents followed by ion-exchange chromatography. The purity of each protein was assayed by SDS-PAGE and gel filtration and its binding properties and biological activity were determined.

**Results** (a) Several mutants of ovine leptins and in one case (R128Q) also human and chicken mutants were prepared. Some of the mutants (T16R, N82W and R12Q) had drastically lower biological activity but only one (R128Q) mutant was antagonistic. However, the antagonistic activity was species dependent. Other mutations (D9R, T12R, D85R/L86F, H89A, A92W) had none or little effect on the biological activity. (b) The library of random mutated analogues of ovine leptin was constructed by error-prone PCR with wild type ovine leptin as a template. The library was inserted into the pAK200 phagemid vector as a fusion protein with pIII minor coat protein of M13 filamentous phage. The resulted protein of interest is expressed as a part of the coat protein of the phage and displayed on its surface. Every single colony of the *E.coli* XL-1 Blue cells transformed with the phagemid was grown to resque the phage particles, which were tested in the BAF/3 cells stably transfected with the long form of human leptin receptor. Wild type ovine leptin displayed in such manner on a phage particle was able to induce the proliferation of the cells. Screening of the phage displayed analogues in the BAF/3 system in the presence of free wild type ovine leptin enables to find analogues with antagonistic activity. (c) A subdomain of human leptin receptor encoding part of ECD (amino acids 428 to 635) was subcloned, expressed and purified to homogeneity. The purified leptin-binding domain (LBD) exhibited predicted beta structure, was capable of binding human, ovine and chicken leptins and formed a stable 1:1 complex with all mammalian leptins. The binding kinetics was assayed by plasmon surface resonance methodology showed respective $k_{on}$ and $k_{off}$ values (mean +/- SEM) of 1.35 x $10^{-5}$ +/- 0.23 $mol^{-1}$ x $sec^{-1}$ and 2.55 +/- 0.50 x $10^{-3}$ $sec^{-1}$ and Kd value of 21.1 +/- 6.5 nM. LBD blocked leptin-induced proliferation of BAF/3 cells stably transfected with the long form of human leptin receptor.

**Conclusion** The present results indicate that the possibility of blocking leptin activity by either, leptin antagonists or LBD is feasible. Further studies directed at optimizing the antagonistic properties of leptin mutants, at increasing the affinity of LBD and at increasing the half-life of both proteins in circulation will facilitate the achievement of this aim.

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# Effect of wilting and type of additive on the fatty acid composition of grass silage

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**Introduction** Forages, such as grass and red clover, are a rich source of *n*-3 polyunsaturated fatty acids, especially α-linolenic acid (C18:3*n*-3), and may be used as a method of improving the nutritional value of ruminant products. Silage is an important feed for cattle, therefore, a study was carried out to elucidate the effects of wilting and the use of additives on the fatty acid profile of the resultant silage.

**Materials and methods** Perennial ryegrass (PRG) was cut on the 24th May 2001 with a hedge-trimmer in the field and chopped twice immediately post-cutting using a garden shredder (Bioline 1100, Atika). The PRG was wilted outside on concrete in bright sunshine (*circa* 25°C) for 0 or 24 h then ensiled in 100ml boiling tubes with water (control, C), Powerstart (bacterial inoculant, P), Ecosyl (freeze-dried bacterial inoculant, E), Addsafe (formic acid inoculant, A) or Quebracho Tannins (QT). Additives were applied at the recommended rate. All treatment combinations were prepared in triplicate. Tubes were left in a closed rack at room temperature and pressure for 104 days, after which the silages were analysed for freeze-dry matter (FDM), lactic acid and volatile fatty acids (VFA). Fatty acids (FA) were analysed by gas chromatography (GC). Results were analysed by general analysis of variance (Genstat 5; Lawes Agricultural Trust, 1995) using the factors 'wilt' and 'additive type', and the interaction.

**Results** Wilting had an extremely marked effect on silage FDM at 18.6 *v.* 66.4 $gkg^{-1}$ (s.e.d.=0.78; P<0.001) for 0 and 24h, respectively. As a result, this reduced the content of lactic acid, 145.1 *v.* 21.1 $gkg^{-1}$ FDM (s.e.d.=4.38; P<0.001) and acetic acid, 5.8 *v.* 1.8 $gkg^{-1}$ FDM (s.e.d.=0.29; P<0.001) for 0 and 24h, respectively. In the fresh material wilting for 24h decreased the content of C18:2*n*-6, 2.7 *v.* 2.4 $gkg^{-1}$ FDM (s.e.d.=0.06; P=0.026) and C18:3*n*-3, 15.7 *v.* 12.8 $gkg^{-1}$ FDM (s.e.d.=0.54; P=0.033) for 0 and 24h, respectively. The proportion of C18:3*n*-3 in the fresh material also decreased after a 24h wilt, 683.2 *v.* 646.2 $gkg^{-1}$ total FA (s.e.d.=0.10; P<0.001) for 0 and 24h, respectively. There was no effect of wilt on the proportion of C18:2*n*-6. Across all additive treatments, wilting reduced total silage FA, 21.3 *v.* 19.4 $gkg^{-1}$ FDM (s.e.d.=0.52; P=0.001) for 0 and 24h, respectively. This mirrored results for the content of C18:2*n*-6, 2.8 *v.* 2.6 $gkg^{-1}$ FDM (s.e.d.=0.05; P<0.001) and C18:3*n*-3, 14.4 *v.* 12.8 $gkg^{-1}$ FDM (s.e.d.=0.43; P=0.001) for 0 and 24h, respectively. The proportion of C18:3*n*-3 decreased with wilting, 683.3 *v.* 646.2 $gkg^{-1}$ total FA (s.e.d.=0.10; P<0.001) for 0 and 24h, respectively. All additives, with one exception, decreased silage FDM at 41.6, 38.6, 49.2 and 38.8 *v.* 44.5 $gkg^{-1}$ FDM (s.e.d.=1.36; P<0.001) for P, E, A, QT and C, respectively. Similar results were noted for the content of acetic acid, 2.9, 4.6, 3.4 and 4.8 *v.* 5.9 $gkg^{-1}$ FDM (s.e.d.=0.50; P<0.001) for P, E, A, QT and C, respectively. Averaged across both wilting times, A and QT led to relatively higher levels of total FA, 21.0 and 22.5 *v.* 20.2 $gkg^{-1}$ FDM (s.e.d.=0.90; P=0.004) for A, QT and C, respectively. QT also increased the content of C18:3*n*-3 at 14.9 *v.* 13.5 $gkg^{-1}$ FDM (s.e.d.=0.75; P=0.013) for QT and C, respectively. There were no interaction effects on the FA parameters (Table 1).

**Table 1** *Effect of wilting (0 and 24h) and additives (C, P, E, A, and QT) on selected silage fatty acids ($gkg^{-1}$ FDM)*

| | 0h | | | | | 24h | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C | P | E | A | QT | C | P | E | A | QT | s.e.d. | sig. |
| FDM/$gkg^{-1}$ | 174.8 | 186.8 | 181.6 | 187.8 | 183.6 | 714.7 | 644.2 | 591.1 | 796.3 | 592.7 | 1.92 | *** |
| pH | 3.7 | 3.6 | 3.6 | 3.6 | 3.7 | 5.8 | 5.6 | 4.9 | 5.7 | 5.1 | 0.25 | *** |
| Lactic acid | 183.9 | 161.6 | 164.3 | 204.6 | 144.7 | 9.5 | 16.3 | 37.3 | 12.1 | 35.7 | 10.73 | *** |
| Acetic acid | 11.0 | 4.3 | 5.4 | 6.4 | 6.6 | 0.8 | 1.5 | 3.9 | 0.4 | 3.0 | 0.70 | *** |
| Total FA | 20.8 | 18.9 | 21.4 | 21.5 | 23.8 | 19.5 | 17.8 | 18.1 | 20.5 | 21.2 | 1.27 | NS |
| C16:0 | 3.1 | 3.0 | 3.2 | 3.2 | 3.5 | 3.0 | 2.9 | 2.9 | 3.2 | 3.4 | 0.16 | NS |
| C18:2*n*-6 | 2.8 | 2.5 | 2.8 | 3.0 | 3.1 | 2.5 | 2.6 | 2.5 | 2.6 | 2.8 | 0.12 | NS |
| C18:3*n*-3 | 14.0 | 12.6 | 14.4 | 14.4 | 16.0 | 13.1 | 11.2 | 11.7 | 13.8 | 13.9 | 1.06 | NS |

NS, not significant, ***P<0.001

**Conclusion** The results of the present study suggest wilting has a significant effect on the fatty acid composition of the resultant silages. Wilting for 24h induced a reduction in total FA and the main individual FA. This may be attributable to oxidation losses and the activity of plant lipases. The biological inoculants tended to have contrasting effects on silage total FA compared with the other additives, which may be related to reductions in in-silo dry matter losses. These results confirm the findings (Dewhurst and King, 1998) that acid additives had no effect on total FA.

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# Odd-chain fatty acids as markers of the microbial colonisation of freshly-ingested grass and microbial contamination of dacron bag residues

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**Introduction** The overall objective of our work is to assess the relative contributions of plant enzymes and rumen microbes to rumen degradation of freshly-ingested herbage. *In situ* techniques have been used extensively to compare rumen degradation characteristics of feeds, though there remain technical problems associated with microbial contamination of residues after incubation. We hypothesised that techniques to study microbial contamination might also provide insights into microbial colonisation. Our earlier studies (Lee *et al*., 1999) identified distinctive odd-chain fatty acids that could be used as microbial markers. A dacron bag study was conducted to examine the influence of dacron bag rinsing techniques on DM disappearance and microbial contamination in residues from fresh grass, assessed using odd-chain fatty acids as markers.

**Material and methods** Two dry Holstein-Friesian cows, fitted with rumen cannulae, were offered a basal diet of perennial ryegrass pasture *ad libitum.* Fresh grass was harvested (07:00 am) and immediately cut to approximately 1 cm lengths (S.D. = 0.50). Sixty grams of fresh grass was weighed into dacron bags (9 x 23 cm; pore size 40 μm) and duplicate bags incubated in each cow for 2, 8 and 24 hours. On removal from the cows, bags were washed by one of 4 different procedures: (1) manual squeezing until the liquid fraction in the bag had virtually disappeared, (2) hand washing, agitating bags in a sink in cold water and repeating until the water in the sink appeared clear, (3) hand washing, under a running tap until water ran clear, and (4) machine washing. Bags were freeze-dried and weighed for dry matter (DM) determination. Fatty acid methyl esters were prepared (methanolic HCl, 5%), extracted and determined by gas chromatography using tricosanoic acid (C23:0) as an internal standard. The effects of rinsing method, incubation time and their interaction were tested by analysis of variance using 'cow' as the blocking factor (residual d.f. = 35).

**Results** The chemical composition of fresh grass was: 112 g DM/kg, 36.5 g total-N/kg DM, 135 g water soluble carbohydrates/kg DM and 33 g ether extract/kg DM. Fresh grass contained detectable levels of three of the odd-chain fatty acids (*anteiso* C15:0, C15:0 and C17:0: 0.21, trace and 0.03 g/kg DM, respectively). Three other odd-chain fatty acids (*iso* C15:0, *iso* C17:0 and *anteiso* C17:0) were not detectable in grass and so were suitable as microbial markers. The different washing procedures influenced DM disappearance in the rumen (Figure 1; SED = 2.82 and $P < 0.01$; SED = 4.28 and $P < 0.05$; SED = 2.51 and $P < 0.01$ for 2, 8 and 24 hours respectively). The concentration of marker odd-chain fatty acids in the residues increased with time of incubation, whilst it decreased with the severity of washing (from method 1 to 4; Table 1).

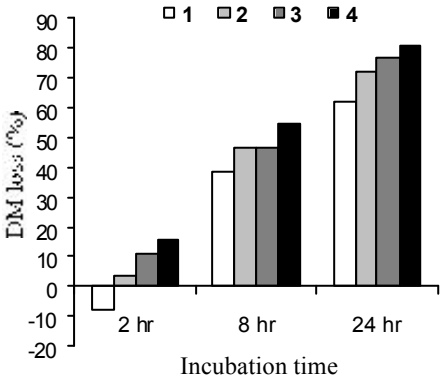


**Figure 1** *Effect of different rinsing methods on DM disappearance (%)*

**Table 1** *Concentrations of odd-chain fatty acids in bag residues (g/kg DM)*

| | | Rinsing method | | | | Sig[1] | | |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | M | T | I |
| *iso* C15:0 | 2 hr | 0.040 | 0.021 | 0.010 | 0 | | | |
| | 8 hr | 0.113 | 0.058 | 0.030 | 0.020 | *** | *** | ***[2] |
| | 24 hr | 0.192 | 0.128 | 0.065 | 0.051 | | | |
| *iso* C17:0 | 2 hr | 0.028 | 0.025 | 0.012 | 0.002 | | | |
| | 8 hr | 0.062 | 0.045 | 0.034 | 0.021 | *** | *** | ***[3] |
| | 24 hr | 0.105 | 0.073 | 0.037 | 0.022 | | | |
| *anteiso* C17:0 | 2hr | 0.081 | 0.066 | 0.048 | 0.040 | | | |
| | 8 hr | 0.227 | 0.152 | 0.105 | 0.080 | *** | *** | ***[4] |
| | 24 hr | 0.445 | 0.263 | 0.150 | 0.110 | | | |

[1]M = rinsing method, T = time, I = interaction
(SED = [2] 0.0097, [3] 0.0070 and [4] 0.0167)

**Conclusions** There were large and highly-significant effects of rinsing techniques on DM disappearance at all incubation times, confirming the technical problems with the dacron bag technique. The significant levels of *anteiso* C15:0, C15:0 and C17:0 in grass precluded their use as microbial markers in this study. However, three other odd-chain fatty acids (see Table 1) were suitable markers, not being present in grass and increasing in line with increasing microbial content in residues (24 hour incubations and no rinsing). Changes in the relative proportions of odd-chain fatty acids may indicate succession by different microbial populations (Lee *et al*., 1999), though this requires further (molecular) validation.

**Acknowledgement** This project was supported by the Department for Environment, Food and Rural Affairs.

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# The relationship between metabolisable energy concentration and nutrient digestibility in grass silages offered to sheep at maintenance

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**Introduction** The energy feeding systems used for dairy cows recommend that the metabolisable energy (ME) concentration of a feed at maintenance feeding level is calculated from its digestible nutrients. The objective of the present study was to develop equations to calculate the ME concentration from digestibility data for grass silages

**Material and methods** A total of 174 grass silages were offered to sheep (4 sheep/silage) as the sole diet at maintenance feeding level to measure nutrient digestibility and urine energy output. The sheep were male Greyface and approximately 2 years old with live weights between 45 and 50 kg; and were housed in individual pens for three weeks before 6 days total collection of faeces and urine in metabolism crates. The silages encompassed primary growth and first and second regrowth perennial ryegrass. The grass was either unwilted or wilted prior to ensiling and ensiled with or without application of silage additives. Gross energy (GE) concentration in silages was determined using undried silages in an adiabatic bomb calorimeter (Gallenkamp, Loughborough, UK). Silage DM concentration was determined on an alcohol-toluene basis, which was subsequently used as a basis of expressing all nutrient concentrations in silages. The silage ME concentration was estimated as the difference between GE intake and energy outputs from faeces and urine and predicted methane energy output (Blaxter and Clapperton, 1965). Linear and multiple regression equations were used to relate silage ME concentration (MJ/kg DM) to its energy digestibility (GED) (MJ/MJ) or digestible organic matter in total DM (DOMD) (kg/kg DM) with GE (MJ/kg DM) or crude protein (CP) (kg/kg DM) concentration of the silage.

**Results** The concentrations of DM, CP and GE in silages ranged from 0.155 to 0.429 (mean 0.227, s.d. 0.0496) (kg/kg), 0.079 to 0.212 (mean 0.137, s.d. 0.0259) (kg/kg DM) and 17.0 to 20.5 (mean 18.6, s.d. 0.64) (MJ/kg DM), respectively. The silages had a wide range of pH (3.49 – 5.49, mean 4.13, s.d. 0.398) and ammonia nitrogen as a proportion of total nitrogen (0.037 – 0.385, mean 0.120, s.d. 0.0643). The regression equations are presented in Table 1 (the values in brackets are s.e. data) and the relationship between ME and DOMD also in Figure 1. All relationships were highly significant ($P<0.001$). In eq. (1a), the constant had no significant effect on the relationship and the omission of this value did not thus influence the $R^2$ (eq. (1b). The latter indicates a value of 15.7 between ME and DOMD and this is close to that (16) suggested in AFRC (1993). The inclusion of CP as a predictor slightly improved the relationship ($R^2 = 0.74$) (eq. (1d)) and the addition of GE as a predictor considerably increased the $R^2$ value to 0.91 (eq. (1c)). The prediction ME concentration using GED, rather than DOMD, had a higher $R^2$ value (eqs. (2a-2c). The $R^2$ value for the prediction using GED and GE as predictors reached to 0.98 (eq. (2c)). The eq. (2b) indicates that ME concentration is 15.1 of GED. There was no significant effect on the relationship between ME and GED with the addition of CP as a predictor, possibly because CP was confounded with GED. The addition of acid detergent fibre concentration as a predictor had no significant effect on either the relationship between ME and DOMD or GED.

**Table 1**. The prediction of ME concentration (n = 174)

| Equations | $R^2$ | |
|---|---|---|
| ME = | | |
| $16.1_{(0.77)}$ DOMD – $0.2_{(0.505)}$ | 0.72 | (1a) |
| $15.7_{(0.070)}$ DOMD | 0.72 | (1b) |
| $15.8_{(0.44)}$DOMD+$0.762_{(0.041)}$GE–$14.2_{(0.80)}$ | 0.91 | (1c) |
| $14.6_{(0.88)}$DOMD+$6.790_{(2.050)}$CP–$0.2_{(0.49)}$ | 0.74 | (1d) |
| $16.9_{(0.51)}$ GED – $1.3_{(0.35)}$ | 0.87 | (2a) |
| $15.1_{(0.05)}$ GED | 0.86 | (2b) |
| $16.0_{(0.21)}$GED+$0.588_{(0.020)}$GE–$11.6_{(0.38)}$ | 0.98 | (2c) |

The unit for DOMD is kg/kg DM

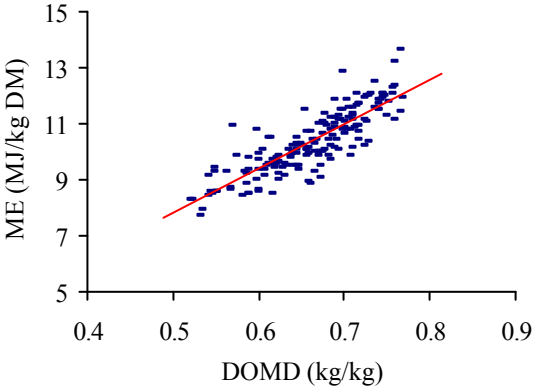


**Figure 1.** ME concentration *v.* DOMD

**Conclusion** The present study reported that silage ME concentration was 15.7 or 15.1 of DOMD or GED and the addition of GE concentration as a predictor considerably improved the relationship between ME and DOMD or GED.

**Acknowledgement** This study was partially funded by DEFRA, MDC and a consortium of industrial partners within a LINK Sustainable Livestock Production project: Feed into Milk.

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# Effects of crossbred ewe genotype and ram genotype on lamb meat quality from the lowland sheep flock

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**Introduction** Ewe and ram genotype have been shown to have a significant effect on carcass quality. For example, Dawson and Carson (2001) demonstrated that lambs from Bluefaced Leicester X Blackface ewes were of poorer conformation than lambs from the Texel X Blackface, Suffolk X Cheviot or Texel Cheviot ewes while high lean growth index sired lambs had an improved conformation compared with lambs sired by non-recorded rams. Recent work has shown that genotype can have significant effects on the meat quality of lambs from hill sheep systems (Carson *et al*. 2001). Therefore the aim of the current study was to investigate the effect of ewe and ram genotype from the lowland sector on meat quality.

**Material and methods** The experiment was carried out over two years on 5 lowland farms located throughout Northern Ireland. Lambs were produced by crossing four breeds of ewe (Blue Leicester X Scottish Blackface (BLXB), Texel X Blackface (TXB), Suffolk X Wicklow Cheviot (SXC) and Texel X Wicklow Cheviot (TXC)) with Suffolk or Texel rams obtained from Sire Referencing Schemes (high lean growth index) or from pedigree sales, selected on the basis of visual appearance (Control). Lambs were slaughtered at 36, 44 and 52 kg. Instrumental meat quality measurements were determined on loin chops taken at 24 h post mortem and measurements of ultimate $pH_U$, cooking loss, Warner Bratzler Shear force and CIELAB colour parameters were determined as outlined by Carson *et al*. (2001). Due to the unbalanced nature of the experimental design, the data were analysed using the Genstat REML (Residual Maximum Likelihood) procedure. This fitted fixed effects for farm and the various ram x ewe breed combinations.

**Results** Lambs sired by Suffolk rams had a higher ultimate pH than lambs sired by Texel rams ($P<0.01$). Lambs sired by high lean index rams were more tender than control sired lambs as indicated by the lower value for Warner Bratzler shear force ($P<0.01$) and this may be related to the higher growth rates observed in lambs sired by high lean index rams (Dawson *et al*, 2001). Ewe genotype had no effect on any parameter of meat quality measured. Irrespective of ewe or ram genotype, when adjusted to a constant conformation (based on the EUROP classification scheme where 1=poor, 5= excellent), significant linear relationships were obtained between fat classification (1= low fat cover, 5 = high fat cover) and $pH_U$ ($pH_U$=5.8–0.03*fat classification $R^2$=0.89 s.e. 0.01; $P<0.05$), a* (a*=12.1+1.37*fat classification $R^2$=0.96 s.e.0.32; $P<0.05$) and C* (C*=14.8+1.62*fat classification $R^2$=0.94 s.e. 0.45; $P<0.05$). Significant linear relationships were also obtained between conformation classification and L* and E* values when results were adjusted to a constant fat class (L*=37.8+0.25*conformation classification $R^2$=0.996 s.e. 0.02 $P<0.05$; E*=42.3+0.39*conformation classification $R^2$=0.999 s.e. 0.01 $P<0.01$).

**Table 1** The effect of ewe breed and ram genotype on meat quality (results adjusted to fat class 3)

| | $pH_U$ | Cooking loss (g/kg raw meat) | Warner Bratzler shear force | L* | a* | b* | C* | E* |
|---|---|---|---|---|---|---|---|---|
| *Ewe breed* | | | | | | | | |
| BLXB | 5.69 | 202 | 2.03 | 38.5 | 15.6 | 11.3 | 19.3 | 43.2 |
| TXB | 5.69 | 203 | 2.20 | 38.9 | 15.9 | 10.7 | 19.2 | 43.5 |
| SXC | 5.68 | 218 | 2.45 | 38.1 | 15.4 | 10.6 | 18.8 | 42.7 |
| TXC | 5.68 | 213 | 2.23 | 37.9 | 16.1 | 11.0 | 19.6 | 42.8 |
| Sem | 0.01 | 7.6 | 0.131 | 0.64 | 0.51 | 0.39 | 0.61 | 0.57 |
| Significance | NS | NS | NS | NS | NS | NS | NS | NS |
| *Ram genotype* | | | | | | | | |
| High lean index | 5.69 | 202 | 2.03 | 38.8 | 15.9 | 11.2 | 19.5 | 43.5 |
| Control | 5.68 | 216 | 2.42 | 37.9 | 15.7 | 10.7 | 19.0 | 42.6 |
| Sem | 0.01 | 5.5 | 0.094 | 0.46 | 0.37 | 0.28 | 0.44 | 0.41 |
| Significance | NS | NS | ** | NS | NS | NS | NS | NS |
| Suffolk | 5.71 | 210 | 2.25 | 38.6 | 15.5 | 10.5 | 18.8 | 43.2 |
| Texel | 5.66 | 207 | 2.20 | 38.1 | 16.1 | 11.3 | 19.7 | 42.9 |
| Sem | 0.01 | 5.3 | 0.091 | 0.44 | 0.35 | 0.27 | 0.42 | 0.39 |
| Significance | ** | NS | NS | NS | NS | NS | NS | NS |

L* lightness, a* redness, b*=yellowness C*(metric chroma)= $(a^{*2}+b^{*2})^{0.5}$, E*=$(L^{*2}+a^{*2}+b^{*2})^{0.5}$

**Conclusions** Ewe and ram genotype effects on meat quality were small and generally non-significant. The significant linear relationships between fat classification and meat quality indicate that this parameter is more important in determining meat quality than any genotypic effects.

**References**

Dawson, L.E.R. and Carson, A.F. (2001). The effect of ewe and ram genotype on lamb output and carcass quality *Proceedings of the British Society of Animal Science, York,* pp. 225

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# The effect of management system at lambing and flock genetics on lamb output on lowland sheep farms

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**Introduction** Systems of lowland sheep production in the UK and Ireland are labour intensive with survey data indicating an average level of labour input of 6.0 to 8.0 hrs/ewe/year (Connolly, 2000). With declining returns from lamb production, management options with reduced labour requirements, such as outdoor lambing, need evaluation. Flock genetics may also influence labour requirements and determine the effectiveness of grass-based lambing systems. Therefore, the aims of current study were firstly to evaluate the effect on lamb output of controlled grass-based lambing systems compared with housing ewes in late pregnancy and lambing indoors and secondly to examine the effect of crossbred ewe genotype and ram breed on lamb output with grass-based and indoor lambing systems.

**Material and methods** The study was carried out over two years on six lowland farms located across Northern Ireland. On each farm the experimental flock (n=90 on average per farm), consisting of Blue-Faced Leicester X Scottish Blackface (BLXB), Texel X Scottish Blackface (TXB), Suffolk X Cheviot (SXC) and Texel X Cheviot (TXC) ewes (detailed by Dawson & Carson, 2000) were divided into three mating groups balanced for ewe genotype, pre-mating condition and age. Ewes in the three mating groups were crossed with high lean growth index Suffolk (n=9), high lean index Texel (n=9) or double-muscled Texel (DM) (n=9) sires. Ewes were also allocated to two lambing systems, balanced for ewe and lamb genotype, ewe age and ewe pre-mating condition score. In the indoor lambing system ewes were housed in late pregnancy and offered grass silage *ad libitum* plus concentrates and ewes and lambs were turned out to grass 1-7 days post-lambing. In the grass-based lambing systems, ewes were removed from the main grazing area in mid-pregnancy and fed supplementary grass silage on cereal stubble ground or indoors. Three to six weeks before lambing, ewes were turned out onto the grazing area with ewes budgeted 2 kg of herbage dry matter per day above a pasture cover of 800 kg DM/ha. At the end of the lambing period, ewes and lambs from the indoor and grass-based lambing systems were grazed together as a single group on each of the farms. The data were analysed using the Genstat REML (Restricted Maximum Likelihood) procedure. This fitted fixed effects for year, ewe genotype, ram genotype, lambing system and the various ewe X ram X system combinations.

**Results** Lambs produced from the grass-based lambing system were heavier at birth ($P<0.05$) however, the level of lambing difficulty was similar in both lambing systems (Table 1). Lambing system had no effect on lamb mortality thus, similar numbers and weights of lambs were weaned from indoor and grass-based lambing systems. Litter size was greater in BLXB ewes ($P<0.001$) compared with the other genotypes. Lamb mortality was not influenced by ewe genotype, while lambing difficulty was lower with SXC ewes compared with BLXB and TXB ewes ($P<0.05$). BLXB ewes weaned a greater number of lambs than the other genotypes and produced a greater weight of lambs weaned per ewe lambed compared with TXC ewes ($P<0.05$). DM-Texel sired lambs had a lower birth weight compared with Suffolk and Texel-sired lambs ($P<0.001$). Consequently the level of lambing difficulty tended to be lower with DM-Texel-sired lambs ($P<0.10$). However, the weight of lambs weaned was lower with DM-Texel sired lambs ($P<0.05$).

**Table 1.** *The effect of lambing system and ewe and ram genotype on lamb output†*

| | No. lambs born/ewe | No. lambs born dead/ewe | Lamb birth weight (kg) | Lambing difficulty score‡ | No. lambs died (birth-weaning) /ewe | No. lambs reared/ewe | Weight of lambs weaned/ewe |
|---|---|---|---|---|---|---|---|
| *Lambing system* | | | | | | | |
| Indoor | 1.79 | 0.10 | 5.0 | 1.28 | 0.10 | 1.59 | 56.8 |
| Grass-based | 1.77 | 0.11 | 5.2 | 1.36 | 0.12 | 1.50 | 56.2 |
| s.e.m. | 0.040 | 0.027 | 0.07* | 0.049 | 0.024 | 0.047 | 1.79 |
| *Ewe genotype* | | | | | | | |
| BLXB | 1.98$^{b}$ | 0.15 | 5.1$^{ab}$ | 1.41$^{b}$ | 0.16 | 1.67$^{b}$ | 61.4$^{b}$ |
| TXB | 1.74$^{a}$ | 0.13 | 4.8$^{a}$ | 1.42$^{b}$ | 0.12 | 1.45$^{a}$ | 54.2$^{ab}$ |
| SXC | 1.74$^{a}$ | 0.11 | 5.0$^{ab}$ | 1.19$^{a}$ | 0.09 | 1.56$^{ab}$ | 56.2$^{ab}$ |
| TXC | 1.64$^{a}$ | 0.09 | 5.2$^{b}$ | 1.37$^{ab}$ | 0.07 | 1.45$^{a}$ | 52.5$^{a}$ |
| s.e.m. | 0.061*** | 0.040 | 0.11* | 0.073* | 0.037 | 0.071* | 2.73* |
| *Ram genotype* | | | | | | | |
| Suffolk | 1.83 | 0.15 | 5.3$^{b}$ | 1.38 | 0.11 | 1.56 | 58.8$^{b}$ |
| Texel | 1.78 | 0.09 | 5.1$^{ab}$ | 1.32 | 0.09 | 1.59 | 58.6$^{b}$ |
| DM-Texel | 1.73 | 0.08 | 4.9$^{a}$ | 1.25 | 0.13 | 1.49 | 52.1$^{a}$ |
| s.e.m. | 0.049 | 0.032 | 0.09** | 0.060 | 0.030 | 0.058 | 2.18* |

*a b Means within columns with same superscripts are not significantly different (P>0.05); †There were no system X ewe or ram breed interactions ‡ 1=no assistance; 5=caesarian section required*

**Conclusions** Lamb mortality rates and lamb output at weaning were similar in grass-based lambing systems compared with labour intensive indoor lambing systems. The relative performance of the ewe and ram genotypes was similar in both systems.

**References**

Dawson, L.E.R. and Carson, A.F. (2001). The effect of ewe and ram genotype on lamb output and carcass quality. *Proceedings of British Society of Animal Science, York,* pp. 225

Connolly, L. (2000). Labour on sheep farms. *Irish Grassland and Animal Production Association Journal. **34:** 112-117.*

# Expected increases in genetic merit in the UK Aberdeen Angus beef cattle breed from applying optimised selection

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**Introduction** Best Linear Unbiased Prediction (BLUP) estimates of breeding values (EBVs) for economically relevant traits have been used for selection decisions in the UK Aberdeen Angus (AA) population since the early nineteen nineties. Selection exclusively based on BLUP-EBVs is expected to give higher gains than less accurate selection but can also lead to increased rates of inbreeding (ΔF). Dynamic rules using BLUP-EBVs to maximise genetic merit while ΔF is constrained to a pre-defined level are currently available (e.g. Grundy et al 1998). They showed that the use of these rules gives higher gains than standard BLUP selection at the same level of ΔF. The objective of this study was to investigate the potential of these procedures for optimising selection decisions in the UK AA population.

**Materials and Methods** Pedigree data and index scores for the aggregate genotype ('Beef Value', derived from BLUP EBVs) were provided by the Meat and Livestock Comission, UK (MLC). The pedigree included 119,953 animals born from 1948 to 2000 with 6,686 male and 38,786 female parents. Selection decisions in 1999 were mimicked and potential candidates (55,553) were males and females born between 1992 to 1998 (according to the observed generation intervals). Only the 7,000 highest EBV ranked animals were included in the optimisations to overcome computing limitations. Optimum contributions were found by using the method described by Grundy et al (1998). Contributions were optimised for both sexes (*opt_all*) and for only male candidates (*opt_males*). In the latter case, female contributions were fixed to *1/2f* where *f* is the number of female candidates. Four levels of pre-defined ΔF were used: 0.002, 0.005, 0.01 and 0.02.

**Results** The observed average ΔF per generation was around 0.002. When ΔF was restricted to the observed value the algorithm tended to allocate similar contributions to selected candidates (Figure 1). Relaxation of the constraint on ΔF (from 0.002 to 0.02) led to unequal contributions with more offspring being allocated to candidates with the highest EBV (Figure 1). The regression of contributions on EBVs was significant ($p<0.01$) for both constraints and increased from $9.8 \times 10^{-4}$ (ΔF =0.002) to $4.3 \times 10^{-3}$ (ΔF=0.02). The expected increase in Index EBV compared with the observed average in 2000 (21.6 units) from applying optimised selection (*opt_all*) was 81.0% (i.e. 39 units) when ΔF was restricted to the observed value (ΔF =0.002, see Figure 2). Less severe restrictions on ΔF led to even higher gains. These increases were achieved by selecting from 68 (ΔF =0.002) to 17 (ΔF =0.02) males and from 81 (ΔF =0.002) to 19 (ΔF =0.02) females, implying unrealistic reproductive rates, particularly for females. When female contributions were fixed, the number of selected males decreased (37 for ΔF=0.002 and 9 for ΔF=0.02), and there was still a substantial expected increase in Index EBV (60.6% for ΔF=0.002 to 69.6% for ΔF=0.02; Figure 2). In practice, losses in selection intensity could occur due to physical unsound or reproductive problems, hence, the benefits from optimised selection could be somewhat optimistic. However, the potential gains from using these tools are clear and very promising.

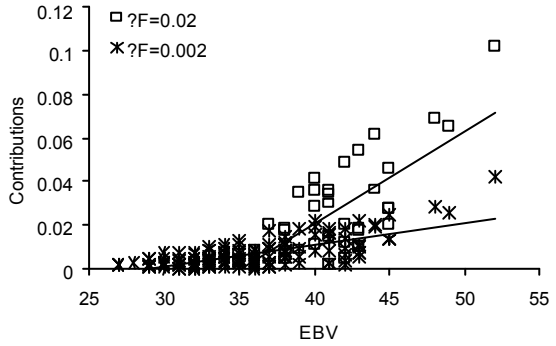


**Figure 1** Relationship between optimised contributions and EBV for two ΔF constraints.

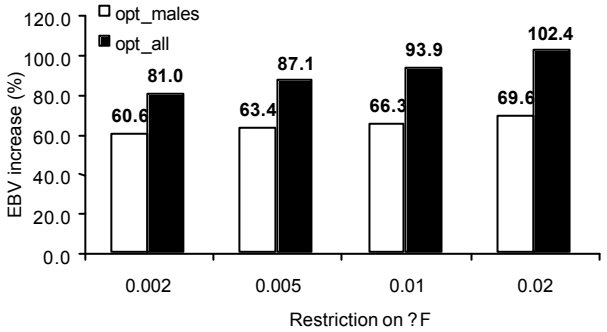


**Figure 2** Percentage increase in Index EBV after optimisation, relative to observed EBV.

**Conclusions** Optimum selection tools could greatly increase the genetic merit of the Aberdeen Angus beef cattle population without concomitant increases in inbreeding. At the observed ΔF, at least 60% increases in average Index EBV are expected. Therefore, even if the current inbreeding rates are not of concern, the use of optimised selection would be highly effective.

**Acknowledgements** Work funded by the MLC through a LINK SLP Project. Roslin Institute receives support from DEFRA and BBSRC. The Aberdeen Angus Cattle Society is acknowledged for allowing use of data. M.Coffey is acknowledged for providing computing support

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# Genetic management strategies for controlling infectious diseases in livestock populations

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**Introduction** Disease resistance is often cited as the major challenge facing animal geneticists, with much effort directed towards finding disease-resistance genes. The PrP gene controlling resistance of sheep to scrapie is such an example. To design effective breeding strategies utilising such genes, it is critical to understand the impact that these genes have upon disease transmission. For example, it has been shown that it is not necessary to make all animals genetically resistant in order to protect the population as a whole from epidemics (MacKenzie and Bishop, 1999). Additionally, concern is often voiced over the possibility of the pathogen co-evolving with the host, reducing the utility of the genes. By combining animal breeding and epidemiology theory, this study derives strategies for using disease resistance genes to control disease transmission, and considers the co-evolution risks with such strategies.

**Materials and Methods** Disease transmission through a population may be summarised by $R_0$, the basic reproductive rate, which describes the number of secondary infections caused by the introduction of an infected animal. If $R_0 > 1.0$ it is expected that a major epidemic will occur but if $R_0 \leq 1.0$, either there will be no epidemic or minor epidemics which die out without intervention. A disease control strategy should aim to reduce $R_0$ to below 1.0, e.g. by mixing genetically resistant animals with susceptible wild-type animals. Assume that for a given disease, when the host population comprises animals with a wild-type genotype, disease transmission is described by $R_{01}$. Now suppose there is a resistance allele, r, such that a population of animals homozygous for this allele will alter disease transmission to $R_{02} < R_{01}$. For a population comprising animals of these two groups, $R_0$ is the weighted average of the rates in the two sub-populations (Dushoff and Levin, 1995), i.e. $R_0 = R_{01}\rho + R_{02}(1-\rho)$, where $\rho$ is the proportion of the population that is wild-type. This can be extended to 3 genetic categories where the proportions of animals are $\rho_0$, $\rho_1$, and $\rho_2$; $R_0 = (R_{00}\rho_0 + R_{01}\rho_1 + R_{02}\rho_2)$. For any combination of genotypes, there are three potential outcomes: no epidemic, a minor epidemic or a major epidemic. Consider now, pathogen mutations leading to co-evolution risks. Mutations will generally cause pathogens to be less fit than wild-type pathogens, thus less able to compete, so co-evolution will mainly be a risk when the mutated pathogen has a competitive advantage over the wild-type pathogen. Thus, co-evolution is not a risk in either wild-type animal populations, or in populations where no animals become infected. If the genetic makeup of the population makes the probability of a major epidemic zero, then the risk of co-evolution is proportional to the risk of a minor epidemic. By algebraic manipulations of the formulae for $R_0$ in genetically mixed populations and extensions to theory describing stochastic epidemic models (Renshaw, 1991), formulae have been derived which describe disease transmission through mixed populations, the impact of errors in the estimation of $R_0$, and probabilities of major or minor or no epidemic, hence, the risks of pathogen co-evolution.

**Results** When resistance is due to a single allele, there are three situations. If $R_{01} < 1$, there is no disease risk. If $R_{02} > 1$, epidemic risks are minimised by having all animals homozygous for the resistance allele. If $R_{01} > 1$ and $R_{02} < 1$, the proportions of the two genotypes should be such that $R_0 \leq 1$, i.e. $\rho \leq (R_0 - R_{02})/(R_{01} - R_{02})$. If populations of resistant animals have $R_{02}=0$, then the proportion of resistant animals must be at least $1-1/R_{01}$. Such populations will not have major epidemics, but can support minor epidemics and still pose a co-evolution risk. Similar, but more complex equations can be derived for the three-genotype scenario, although these equations have non-unique solutions, i.e. the breeder has flexibility in the proportions of each genotype of animal in the population. For example, in the situation where $R_{00}=5.0$, $R_{01}=2.5$ and $R_{02}=0.0$, a solution is $\rho_0$ and $\rho_1=0.067$, and $\rho_2= 0.867$. When $R_0 \leq 1$, the co-evolution risk is proportional to the risk of a minor epidemic, which can be shown to be $R_0/(R_0+1)$, i.e. 0.5 at the threshold $R_0=1$. The relative risks of minor epidemics for different $R_0$ values are shown in Figure 1. Now consider when $R_0$ values are imprecisely estimated. Assume that $R_{02}$ is underestimated by a proportion d, but $R_{01}$ is known accurately. Let $R_{02}^* = R_{02} + dR_{02}$, the true $R_0$ in the resistant population. The expected $R_0$ is now $R_0^* = \rho R_{01} + (1-\rho)R_{02}^*$ and the probability of a minor epidemic is $(R_0 + dR_{02}(1-\rho))/(R_0 + dR_{02}(1-\rho)+1)$, when $R_0^* < 1.0$. Therefore, underestimating $R_{02}$ increases the probability of a minor epidemic and hence the risk of co-evolution.

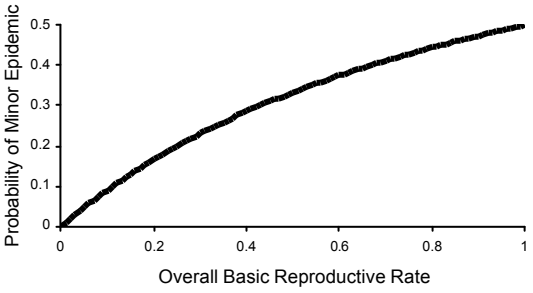


**Figure 1.** Probability of minor epidemics as a function the basic reproductive rate ($R_0$).

**Conclusions** Methods have been presented to effectively utilise disease resistance genes. It is not necessary to make all animals resistant, but the required proportions of resistant animals depends upon the relative $R_0$ values of each genotype and the accuracy with which these have been estimated. Pathogen co-evolution is a risk when minor epidemics can occur; this is a function of the overall $R_0$. These formulae will assist breeders to effectively utilise resistance genes.

**Acknowledgements** We gratefully acknowledge BBSRC for funding.

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# Comparison of deterministic and stochastic methods of calculating identity-by-descent matrices using multiple markers

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**Introduction** Identity-by-descent (IBD) matrices are used for a number of practical applications, e.g. QTL-detection, marker assisted selection in breeding schemes (MAS), refining of covariances among relatives, and MAS for maintaining genetic variation. The calculation of IBD matrices can be made using Markov Chain Monte Carlo (MCMC). However, this is a computationally expensive method. Therefore, a simple deterministic method (Det) has been developed (Pong-Wong *et al.*, 2001). The objective of this study is to evaluate this deterministic method relative to MCMC for the precision of the matrices and their performance in interval mapping and MAS.

**Materials and methods** 4 generations were simulated following a base population. From each generation 5 males and 10 females were selected randomly, and mated hierarchically. Litter size was 10, i.e. 5 of each sex. Three types of markers were simulated: (i) Single Nucleotide Polymorphisms (SNP), i.e. biallelic markers with allele frequencies 0.9 and 0.1 (2U); (ii) Biallelic markers with intermediate allele frequency (2E); and (iii) Microsatellites, i.e. markers with 8 alleles of equal frequency (8E). Three densities of marker maps for a chromosome of 105 cM were used: markers for each 3, 7, or 15 cM. IBD evaluations were performed at the position 52.5 cM, where the true IBD status was known from the simulations. IBD matrices were calculated using Det, MCMC, as implemented in Loki (Heath, 1997), and using pedigree information only. The precision ($E_R$) of Det relative to MCMC was calculated using the Mean Squared Error (MSE), i.e. the deviance from the true IBD status, and corrected for the contribution of the pedigree. For 4 scenarios the matrices were also used in interval mapping (George *et al.*, 2000) and MAS (Fernando and Grossman, 1989), where the performance was tested using the Likelihood Ratio test statistic (*LR*) and the accuracy of prediction of QTL-effects (*r*), respectively.

**Results** The results from interval mapping and MAS for four scenarios are given in Table 1. Increasing frequency of the rarer of two alleles and increasing density of marker map results in increased test statistics and accuracies. However, the ratio of test statistics or accuracies for Det and MCMC shows that Det is doing best for situations with rare alleles relative to MCMC. This is also clear from Figure 1, which shows the relative precision for all 9 scenarios. 2U's are always better than 2E's, even though there is less information from the markers. For 8E, the relative precision is very close to 1.

**Table 1** Average of 50 replicates of results from interval mapping and MAS for combinations of two marker types and two densities of marker maps, and the relative precision of Det.

| Markers | | *LR*[#] | | | *r*[*] | | | |
|---|---|---|---|---|---|---|---|---|
| Type | cM | MCMC | Det | Ratio | MCMC | Det | Ratio | $E_R$ |
| 2E | 3 | 11.4 | 10.5 | 0.92 | 0.65 | 0.63 | 0.96 | 0.88 |
| 2E | 15 | 7.02 | 6.47 | 0.92 | 0.55 | 0.51 | 0.93 | 0.75 |
| 2U | 3 | 9.15 | 8.64 | 0.94 | 0.59 | 0.59 | 0.99 | 0.95 |
| 2U | 15 | 3.65 | 3.26 | 0.89 | 0.45 | 0.45 | 1.01 | 0.90 |

[#]s.e.m. of *LR*: 0.5-1.2
[*]s.e.m. of *r*: 0.015-0.02

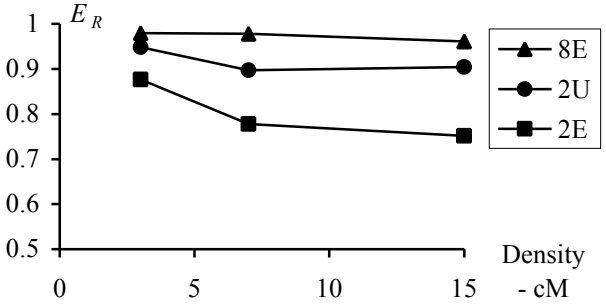


**Figure 1** Effect of density of marker map on $E_R$.

**Conclusions** The results from this study show that the deterministic method can be used as an alternative to MCMC in situations where highly polymorphic markers, e.g. microsatellites, are used, independent of the density of marker map. It is also performing very similar to MCMC for a dense marker map of SNP's. However, for sparse marker maps with biallelic markers, the relative precision of Det is less than 0.95, due to an incomplete use of the marker information.

**Acknowledgements** Roslin Institute gratefully acknowledges funding from DEFRA and BBSRC. JJW gratefully acknowledges funding from SENTER (BTS-project) and Holland Genetics.

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# Benefits from marker assisted selection with optimised contributions and prior information on the QTL effect

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**Introduction** Studies investigating the value of Marker Assisted Selection (MAS) for increasing genetic gain have compared responses from MAS and conventional schemes obtained with standard truncation selection and have ignored rates of inbreeding, ΔF (e.g. Ruane and Colleau, 1995). On the other hand, research comparing schemes at the same ΔF using optimised selection (Villanueva *et al.* 1999) has assumed that the effect of the QTL is known without error. This study extends the optimisation methods to include selection on genetic markers rather than on the QTL itself.

**Methods** A trait controlled by polygenes and a single biallelic additive QTL was simulated. The polygenic ($s_u^2$) and environmental ($s_e^2$) variances were 0.2 and 0.8, respectively. The effect of the QTL, defined as half the difference between the two homozygotes, was $0.5s_p$ ($s_p^2 = s_u^2 + s_e^2$) and the initial frequency of the favourable allele was 0.15. Two polymorphic markers flanking the QTL at distance $d$ (in cM) were simulated. The number of candidates per generation was 120. Schemes compared for rates of gain at a fixed ΔF (5%) were: 1) PHE: selection ignoring genotype information; 2) GAS: direct selection on the QTL; and 3) MAS: selection on the markers. BLUP optimised selection (Villanueva *et al*., 1999) was used in all cases. The genetic evaluation in MAS was carried out following Fernando and Grossman (1989). The benefits from MAS were also investigated when independent prior information about the QTL effects was used to increase the accuracy of the estimates. The prior information was included into the MAS evaluation by adding information of ($n$) 'phantom' offspring into the mixed model equations. Only the equations related to the QTL effects in the mixed model were modified. Different accuracies for the prior, referring only to the QTL (0.14, 0.40, 0.81 and 0.98, corresponding to $n$ = 1, 10, 100 and 1000, respectively) were considered.

**Results** Figure 1 shows responses from GAS and different MAS scenarios varying in $d$, expressed as a deviation from the gain achieved with PHE. The narrower was the marker bracket, the closer was the response to selection from MAS to the response from GAS. However, even for the smallest distance between the QTL and each marker ($d$ = 0.05 cM), MAS achieved only a small proportion of the extra gain obtained with GAS in the early generations. The maximum accumulated benefit in MAS over PHE (observed at generation 3), was, at most, half of the maximum benefit achieved by GAS (observed at generation 2). The use of prior information on the QTL increased substantially the potential of MAS (Figure 2). Even with priors of low accuracy there was a clear improvement in the response compared to that obtained from standard MAS ($n$ = 0). The use of priors with high accuracy led to responses as high as those with GAS.

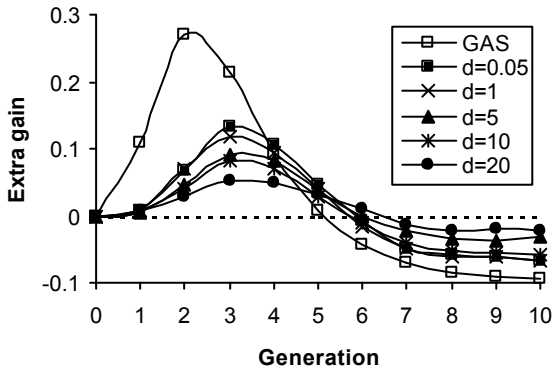


**Figure 1.** Extra genetic gain ($s_p$) relative to PHE from GAS and MAS with different $d$ values

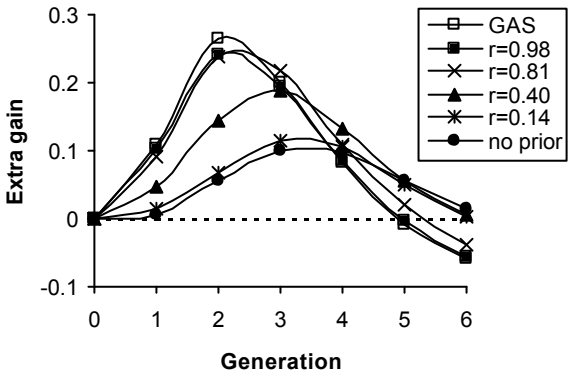


**Figure 2.** Extra genetic gain from MAS ($d$ = 10 cM) using prior information with different accuracies (r)

**Conclusions** The use of markers leads to moderate short-term extra gains relative to schemes PHE. However, the use of prior information on the QTL's effects can substantially increase genetic gain, and, when the accuracy of the prior information is high enough, the responses from MAS are practically as high as those obtained with GAS.

**Acknowledgements** This work was funded by BBSRC, PIC, Genus-Holland Genetics and MLC through the LINK SLP Programme and by SEERAD and DEFRA. We thank S. Avendaño for his comments.

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## Predicting inbreeding using markers and long-term genetic contributions

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**Introduction** Quantitative Trait Loci (QTLs) are chromosomal regions containing genes causing variation in continuous traits. Two alleles are Identical-By-Descent (IBD) if they are copies of the same original allele. For each point on a chromosome, it is possible to predict IBD probabilities among individuals given a pedigree and marker information and use them to map QTLs in farmed species. Pedigree founders can either be assumed unrelated (George et al. 2000) or related through distant common ancestors (Meuwissen and Goddard 2000). The relationship among founders was estimated through simulating the history of a model population. Linking the current population with original ancestors, without a pedigree, is central to the theory of long-term genetic contributions (Bijma 2000). Hence, in the light of this theory, it should be possible to deterministically predict IBD relationships among pedigree founders. A first step towards this objective was predicting inbreeding (F) within each animal at a chromosomal location.

**Materials and methods** An estimator of the population inbreeding coefficient between any two loci (shared inbreeding Fs) is $F_s = 1/4 \sum_i r_i^2 \sum_{t=0}^{T} (1-\Delta F)^t (1-2C)^t$ [1], which simplifies to the single locus inbreeding (F) when C=0, $F_s = F = 1-(1-\Delta F)^T$ [2]. Here, $r_i$ is the long-term genetic contribution of the $i^{th}$ ancestor to the present population, T is the total number of discrete generations separating ancestors from the current population, ? F is the rate of increment of inbreeding per generation, and C is the recombination rate between loci. The inbreeding coefficient of animal i at chromosome location p was predicted via the multiple regression $F_{ip}$=F+B'X+e [3], where X is a vector of corrected Identity-By-State (IBS) values for all marker loci, and B is a vector of regression coefficients relating IBS at each marker locus with IBD at location p. The simulation comprised a population of size 50, initially in linkage equilibrium, evolving through 100 discrete generations of random mating including selfing.

**Results** Figure 1 shows how expectations obtained with [1] match very accurately simulation results for 3 different values of C. Figure 2 shows the Prediction Error Variance around true F (PEV($F_{ip}$)) when individual inbreeding at position p is obtained using 0, 1 (C=0.001) and 4 (C=0.2, 0.1, 0.01, 0.001) marker loci. There is a gain in accuracy (i.e. lower PEV($F_{ip}$)) when Fs is predicted using marker information, as opposed to using none (i.e. using formula [2] ).

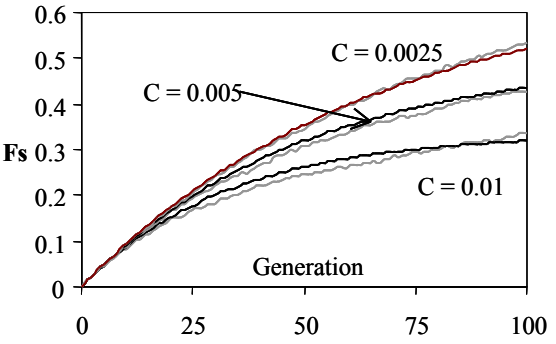


**Figure 1. Expected (dark) and simulated (grey) Fs given the recombination rate C**

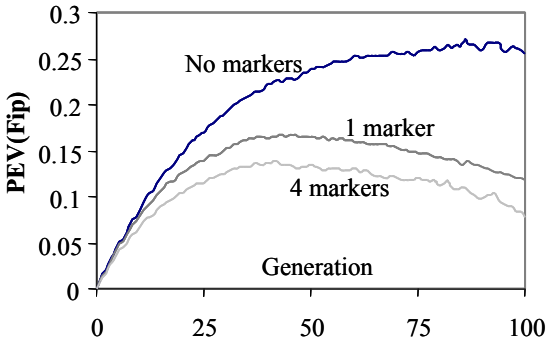


**Figure 2. PEV(Fip) using 0, 1 or 4 markers**

**Conclusions** This study shows that formula [1] is predicting Fs as well as simulations for the set of scenarios considered here. Hence, [1] was incorporated into a multiple regression model [3] to predict individual inbreeding using marker information. PEV($F_{ip}$) can be reduced by using marker information. Further work will extend this approach to obtain point IBD probabilities between animals.

**Acknowledgments** This work is supported by PIC and BBSRC

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## Genetic lag in a Meishan x Large White pig backcross population: A simulation study

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**Introduction** Backcrossing can be used as a tool to introduce new alleles into a population. Having detected an allele of interest in a non-commercial (donor) line, backcrossing methods introduce the allele into a commercial (recipient) population whilst minimising the contribution of the less superior donor genome. Many alleles linked to the desired donor allele are incorporated into the recipient line by a phenomenon called linkage drag. Loci in the region of the target locus may trace back to a common ancestor and become identical by descent (IBD). This leads to a loss of diversity around the target locus. The linkage drag and contributions from ancestral recipient populations mean that the backcross population suffers genetic lag for commercial traits. This study aims to investigate the effect of population size and number of backcross generations on genetic lag, linkage drag and IBD around a target allele reducing back fat found in the Chinese Meishan breed when backcrossed to a commercial Large White population.

**Materials and Methods** The initial cross assumed *N* donor Meishan boars were mated to *N* Large White sows with an average of eight piglets per litter throughout backcrossing. At each backcross generation *N* carriers of a QTL allele on pig chromosome 7 at 58 cM (additive effect of decreasing fat depth at shoulder, FS, by 2.7 mm, Walling *et al*, 1998) were selected. Meishan and Large White differed by approximately 280 g (679 vs. 395) for average daily gain (ADG) (Haley *et al*, 1992) with a rate of genetic progress in commercial lines of 15 g in ADG per annum (A.D. Hall *pers comm*). Chromosome 7 is 150 cM and the size of the pig genome is 25M. The methods of prediction of linkage drag and genomic contributions post introgression were as described by Wall *et al* (2001). Genetic lag (difference between backcross and commercial population in generations of selection) in ADG was measured by weighting genomic contributions by the genetic worth of each. Simulation studies were used to examine IBD around the target locus at the end of backcrossing. The number of parental pairs (*N*=10-100) and the number of backcross generations in the introgression scheme (*T*=1-10) were varied in the simulation studies and run for 500 replicates.

**Results** Genetic lag decreased as the number of backcross generations rose (Figure 1). Changes in population size had no significant effect on genetic lag (results not shown). Linkage drag decreased and proportion of loci IBD within the linkage drag increased as backcross generations increased (Figure 2). The proportion of loci IBD on the carrier chromosome was relatively constant over backcross generations (~0.03 when *N*=20). Proportion of loci IBD decreased as population size increased (results not shown). IBD on the carrier chromosome was mainly due to the high levels around the target locus and this occurred due to the limited number of contributions from donor ancestors.

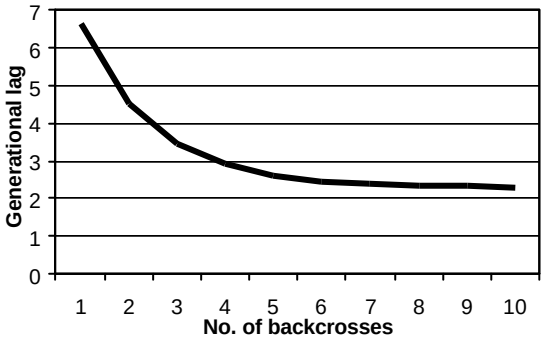


**Figure 1:** Effect of the number of backcross generations (*T*) on genetic lag

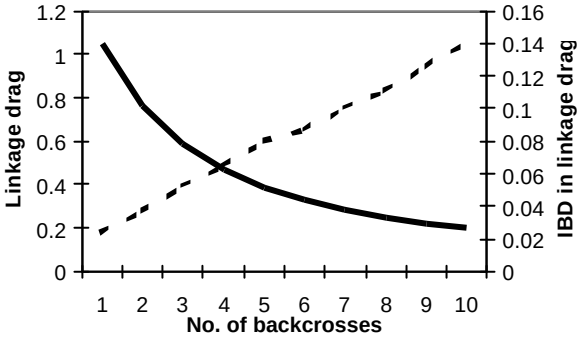


**Figure 2:** Effect of *T* on linkage drag length in Morgans (solid line) and loci IBD in the linkage drag (dashed line)

**Conclusions** The results show genetic lag reduces in early backcross generations but after 5 generations the lag approached an asymptote. Although there is genetic lag for ADG, the beneficial effect of the back fat allele may compensate for this lag. For this scheme it appeared that it was necessary to have no more than 5 backcross generations and 80 mating pairs at each generation to maintain an acceptable level of genetic lag and IBD around the target locus but this has to be balanced by an acceptable level of genetic lag. The methodology presented can be used to help design breeding schemes that achieve the goal of introducing a beneficial allele into a recipient population whilst minimising undesirable parameters in a breeding population post introgression.

**Acknowledgements** Eileen Wall was funded by a Teagasc Walsh Fellowship, Irish Cattle Breeders Federation and Ministry of Agriculture, Food and Fisheries UK.

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# Comparison of available and required metabolisable energy (ME) resources for livestock during winter in an agro-pastoral system of the Hindu Kush – Himalayan region of Pakistan

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**Introduction:** The semi-arid Hindu-Kush-Karakoram-Himalayan region of Pakistan covers 72,000 sq km with a rainfall of 100-400 mm per year[5]. Limited arable land and water scarcity have made subsistence farming the dominant agro-pastoral farming system. Each household keeps a range of ruminant livestock species such as goats, cattle, sheep, donkeys and yaks their proportion in the herd are 0.53, 0.23, 0.19, 0.03 and 0.02 respectively. In winter, livestock are confined and stall-fed on stored roughages or grazed on marginal lands and fallow agricultural fields close to the villages. The aim of this study was to quantify nutritional inputs in terms of metabolisable energy resources, and to compare these with ME requirements of the animals for maintenance over winter.

**Materials and Methods: A 2 x 3 factorial design was used where factors were transects at t**wo levels (i.e. Karakoram Highway (KKH) and Gilgit Ghizar Region (GGR) and agro-ecological zones (AEZ) at three levels (i.e. single, transitional and double cropping zones). Transects were selected on the basis of their transport infrastructure and access to markets. The KKH transect had a good road infrastructure and the GGR transect had a poor one. Within each transect one village/AEZ was selected. Six or seven households (hh) per village were selected for study using a stratified random sampling technique. Stored feed resources were estimated for each hh at the start of winter by physical measurement of their volume and density. Total stored energy per hh was calculated using published values of ME[6] of the feeds. Liveweights of all livestock in the selected hh were measured at the start of winter to estimate metabolic liveweight ($kg^{0.75}$). ME requirements for liveweight maintenance were calculated using AFRC guidelines (1993[7]). Sufficiency of ME was calculated by dividing the ME in the stored feeds per hh by the estimated ME requirements of the animals per hh over winter. Data were analysed using the REML procedure of Genstat 5.

**Results**: Feed sufficiency in terms of the ME for liveweight maintenance in the stored feed was significantly higher ($P < 0.05$) in the KKH transect compared to the GGR transect (Table 1). Feed sufficiency did not differ according to AEZ. Feed sufficiency values of less than 1 indicate feed resources were insufficient to maintain liveweight. For example in the single cropping zone village of the GGR transect, estimated ME required was 74.6 MJ, whereas available ME was 48.2MJ,indicating a proportional shortfall in feed resources of 0.4.

Table 1. Feed sufficiency for maintenance over winter (150 days), ME required per $kg^{0.75}$ and available stored feed resources in the study households

| Parameter | Transect | Single | Transitional | Double | Mean | P-Value |
|---|---|---|---|---|---|---|
| Feed sufficiency ratio | GGR | 0.6±0.09 | 1.2±0.09 | 0.6±0.12 | 0.8±0.08 | |
| | KKH | 1.3±0.14 | 1.0±0.08 | 1.2±0.38 | 1.2±0.12 | 0.007[c] |
| | Mean | 1.0±0.13 | 1.1±0.06 | 0.9±0.19 | | |
| | P-Value | | 0.535 [a] | | | 0.024[b] |
| ME required/ $kg^{0.75}$ /hh (MJ) | GGR | 74.6±1.59 | 75.9±1.05 | 78.6±1.20 | 76.5±0.79 | |
| | KKH | 65.0±2.04 | 76.8±2.82 | 79.4±0.72 | 73.3±1.87 | 0.021 [c] |
| | Mean | 69.4±1.86 | 76.3±1.36 | 78.9±0.70 | | |
| | P-Value | | 0.001 [a] | | | 0.002[b] |
| Stored ME/ $kg^{0.75}$ /hh (MJ) | GGR | 48.2±6.6 | 85.2±8.1 | 47.9±9.75 | 61.7±6.17 | |
| | KKH | 99.4±4.05 | 72.4±6.05 | 92.8±27.1 | 88.2±9.75 | 0.015[c] |
| | Mean | 73.8±9.8 | 79.3±5.3 | 70.4±15.3 | | |
| | P-Value | | 0.744 [a] | | | 0.027[b] |

values are presented as mean± SEM [a]agro-ecological zone effects, [b]transect X zone interaction effects, [c]transect effects

**Discussion**: Livestock appeared to be better maintained over winter in the KKH transect. This may be associated with a better transport infrastructure, because better roads provide not only easy access for imported feeds, but also itinerant contractors could make frequent visits to purchase animals, allowing households to minimise their flock according to available resources.

**Acknowledgements:** The project was funded by the European Union-INCO DC Programme

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# Sheep productivity in private flocks in Kazakstan

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**Introduction** During the Soviet era, state or collective farms on the rangelands of Kazakstan moved livestock between winter, spring, summer and autumn pastures in different ecological zones. By the end of the Soviet era, livestock production was still based partly on the traditional migratory system, but had become heavily dependent on supplementary winter feed over the harsh winters. When the majority of former state and collective farms became cooperatives in the mid 1990s, they retained the same management structure, but individuals became members of the cooperatives or had the right to a share of the assets (e.g. livestock, machinery, access to land) and become private farmers. Many individuals, who remained cooperative members and are not private farmers, still keep private livestock. There are, in addition, an increasing number of private farmers. Most flock owners cannot now afford to follow a four-season migratory system of sheep management nor to obtain sufficient winter feed of good quality. Small-scale flock owners with less than about one hundred sheep lack the resources (e.g. family labour, transport) to move animals the long distance between the different pastures. There has been a large decrease in sheep numbers and there is now excess pasture in all ecological zones, as many sheep now spend the whole year within 10 km of the home village or private farm. However they need to be supplied with winter fodder since the vegetation ceases to grow in autumn and is often covered in snow from December to March. The aim of the present study was to determine current management practices and their effects on sheep productivity.

**Materials and methods** During 1998 and 1999 semi-structured interviews were conducted with 18 sheep flocks owners in the semi-desert and steppe areas in Jambul Raion, Almaty Oblast. Information was collected on the structure, productivity and nutritional management of the flocks. To explore further the relationship between flock productivity and winter nutrition, detailed information on winter feeding practices was obtained from seven flocks.

**Results** Flocks were a mixture of Kazak fine-wooled sheep and Kazak fat-rumped sheep. Lambing typically occurred in February or March. All flocks were kept indoors and fed hay in winter (from late November or December until March, depending on the weather), in some cases they grazed pasture during the day and were turned out to pasture in March (depending on the weather), and grazed around the village on natural pasture. In summer all the sheep were kept within a few kilometres of the village or barn in the steppe or semi-desert area. Body condition score was highest in October and lowest in April (Table 1). Of the seven flocks studied in detail, some were offered hay *ad libitum* while others were restricted. Three flocks were fed concentrate, usually barley, in winter. One of these livestock owners grew barley, while the other two bought or bartered for concentrate, and fed from 200 to 500g of barley per ewe per day in winter. Those flocks that received concentrates in winter had higher numbers of lambs born per ewe (Figure 1). The better performing flocks also tended to be the larger flocks. The mean flock size for the flocks receiving concentrates was 148 compared to 18 for those not fed concentrates.

**Table 1.** Flock productivity

| | Mean | s.d |
|---|---|---|
| Body condition score | | |
| January | 2.18 | 0.317 |
| April | 1.36 | 0.136 |
| October | 2.56 | 0.093 |
| Lamb born (per 100 ewes) | 119 | 24.7 |
| Lamb mortality (per 100 ewes) | 10.3 | 8.59 |

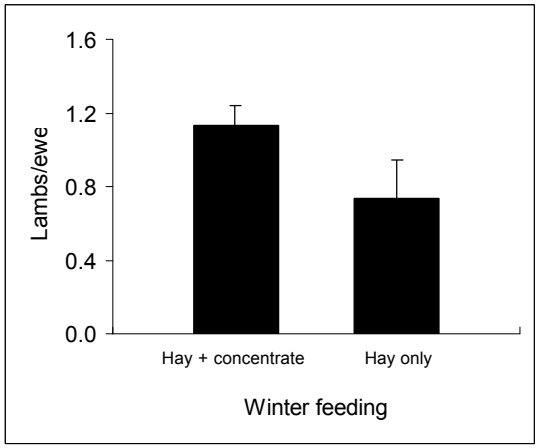


**Figure 1.** Winter feeding and lambs born

**Discussion** The results suggest that the output from the system is sensitive to the level of winter nutrition, since flocks which were fed concentrates in winter achieve higher levels of animal performance. Owners of small flocks lacked the resources to be able to acquire concentrate feed and good quality hay. The number of sheep in these small flocks was decreasing because the family needed to slaughter sheep for consumption, barter sheep for household inputs and therefore could not produce sufficient replacements to maintain the flock size. The larger flocks tended to be increasing in size since there were sufficient lambs produced as breeding replacements even after slaughter for consumption and selling or barter.

**Acknowledgements**
This work was funded by the Department for International Development. However the views and opinions expressed here do not reflect DFID's official policies or practices, but are those of the authors' alone.

# Farmers' evaluation of five technologies that enhance sheep productivity in West Asia

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**Introduction** Attempts to improve the productivity of farm animals in developing countries have often been thwarted by the inappropriateness of the technologies to the conditions in the recipient countries. The reasons for the successes and failures are numerous, among them the degree of participation of the client farmers testing technologies, government policy, the strengths of the extension service and the prices of inputs and outputs. A study was conducted between 1990 and 1995 in Iraq, Jordan and Syria that aimed to assess the potential for adoption of a number of technologies which were known to improve sheep productivity. The technologies were tested at the farm level under the supervision of researchers in the three national programs.

**Materials and methods** Sheep-owning farmers growing rainfed crops such as barley, wheat and food legumes were selected in the semi-arid areas of Iraq, Jordan and Syria where the annual precipitation is 250 to 350 mm. The mean number of sheep owned by the 154, 201 and 101 farmers in Iraq, Jordan and Syria was, respectively, 324 (s.e. 37.8), 260 (25.6) and 84 (10.9). One or more of each of the following technologies were tested on the flocks: urea-treated straw (UTStraw), multi-nutrient feed blocks (FBlock), oestrus synchronisation with super-ovulation (OSSO), vitamin ADE injections (VitADE) and early weaning of lambs (EWean). The samples of farmers were divided into two groups. First, a control group who were not involved in testing the technologies. Second, participating farmers who hosted tests on one or more of the technologies, attended field days about one or more of the technologies, or both. In 1996 all the farmers were interviewed using a structured questionnaire to evaluate their attitudes about the technologies. A sub-sample of the second group were asked how often they had applied the technologies and whether they intended to use them in the future. The sub-sample was categorised as either Rejecters or Innovators depending on whether they had, respectively, not used the technology or used it at least once after hosting tests or attending field days. The farmers' responses to the questions were evaluated using chi-squared in a 'three countries x two categories' contingency table. Only the results from the participating farmers are presented here.

**Results** Although the values of $\chi^2$ were influenced by differences in the size of the samples across the three countries, there was evidence of some variation in the evaluation of the technologies by the farmers (Table 1). The numbers rejecting and innovating with UTStraw and EWean were similar but more farmers rejected OSSO and VitADE than were innovating with them. However, the opposite was found with FBlocks, with more farmers trying the technology than rejecting it. Proportionately 0.16, 0.20, 0.22 of the farmers who had tested or had seen, respectively, OSSO, VitADE and EWean later used them at least three times. When the rejecters were asked whether they intended to continue using the technologies at some later date, proportionately 0.8 to 1.0 of them said they would. This may be evidence that the farmers' response was designed to please the interviewers. Proportionately 0.63 of the sample in Iraq owning more than 300 sheep gave flock movements as a reason for not using UTStraw whereas in flocks of less than 100 head this was not a problem ($\chi^2$ = 13.3, P<0.001). The availability and price of the inputs was a key issue regarding attitudes towards OSSO and VitADE. Although farmers in Jordan felt that EWean enhanced flock productivity, high labour needs and high lamb mortality were constraints.

Table 1. Numbers of farmers classified as rejecters or innovators who had tested or observed the technologies.

| Technology | Rejecter | Innovator | $\chi^2$ | P |
|---|---|---|---|---|
| UTStraw | 25 | 26 | 3.7 | ns |
| FBlock | 17 | 38 | 41.0 | <0.001 |
| OSSO | 66 | 48 | 10.5 | <0.01 |
| VitADE | 58 | 39 | 21.0 | <0.001 |
| EWean | 15 | 26 | 1.2 | ns |

**Conclusions** The results from the survey show once again that farmers in semi-arid regions are extremely cautious about adopting new technologies. The survey also highlighted the difficulties of evaluating farmers' perceptions of technologies when the duration of a project is even five years. Moreover, farmers' evaluation of technologies can be biased during interviews. It is essential to conduct follow-up surveys two or three years after the end of projects to assess what proportion of the innovators continue to use the technologies.

**Acknowledgement** The studies reported here were given generous support by the International Fund for Agricultural Research (IFAD) and the Arab Fund for Economic and Social Development (AFESD). Thanks are expressed to K. Shideed, A. Haboobi, I. Kreisat, K. Mamdouh, Y. Swaidan and A. Kneifis who supervised the field work and collected the data.

# Enzymes in Poultry Diets.

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**Poultry diets and production** Poultry diets in northern Europe are composed of relatively high quality ingredients, consisting primarily of wheat and soyabean meal with smaller quantities of barley, peas, beans, rapeseed meal and sunflower meals. The cereal component is usually from the less expensive residual material from human use and as a result the quality of the ingredients tend to vary in nutritional value. The major cost of production of poultry resides with the diet (around 60-70%). Thus, strategies that reduce dietary costs, and increase the efficiency of utilisation of nutrients have important implications in the production of poultry. The major types of poultry production in Europe consists of chicken, turkey and laying hens (6.2Mt chicken meat, 1.8Mt turkey meat, 5.1Mt eggs, from a total of 33.9Mt of meat). Thus poultry products, including eggs, accounts for about 39% of meat products and therefore assumes significant financial importance (FAO, 2002).

An important method of reducing dietary cost is to include poorer quality ingredients in the diets. These ingredients usually have higher quantities of 'fibre', lower quantities and availability of proteins and amino acids and have poorer ME values. This usually has the effect of reducing nutrient content and availability in the diet and thus reduces performance and homogeneity of product. Enzymes are included in more than 90% of poultry diets in northern Europe and thus are an important factor in poultry production. The enzymes of most importance are the carbohydrases, proteases and phytases and often these are present in enzyme supplements with combined activity (EU, 2001, Anonymous, 2001). A major factor for inclusion of enzymes in poultry diets within the EU is that they must have demonstrable beneficial effects such that they can be registered for use within the EU. Beneficial effects are more easily seen in the young bird rather than the older bird, which is presumably due to the poorer functional ability of the GIT in the young chick (Sklan, 2001). Large efforts and expenditure are made to obtain data that show beneficial effects in terms of performance, nutritional value and animal health in relatively small numbers of animals. Conversely, manufacturers must demonstrate under rigorous conditions, that excess enzyme (at least 10 times recommended levels) causes no adverse effects (EU, 1999). One of the major problems in studies on the efficacy of enzymes in poultry diets is the analyses of the enzymes in the completed diets to assure that these have been added at the correct concentrations or have not been deactivated during manufacture of the diets. This has been a major difficulty in attempts to assure enzyme presence but in some respects, not surprising. The ability of enzymes to function effectively is assured by their strong interaction with their substrates. Thus the ability of analysts to extract the supplementary enzymes from diets is likely to be due in part to their interaction with their respective substrates (McCleary, 2000).

It is well known that enzymes have different effects at different stages of growth, which will be due, in part, to the different diets provided throughout the production cycle. It is likely that these differences are due to a combination of both diet and age as well as adaptation of the gut and the microflora therein (Sklan, 2001). Variable effects can also be due to the effects of the enzymes and dietary constituents on endogenous losses (Cowieson *et al*, 2002;). It has been demonstrated that the ingestion of NSPs, protease inhibitors and tannins increase the loss of endogenous materials from animals. Furthermore it has recently been demonstrated that enzyme ingestion can increase endogenous losses from chickens (Cowieson *et al*, 2002).

**Conclusions** Thus, for enzyme supplementation of poultry diets to be effective, diets must be designed to be nutritionally sub-optimal, and the quantity (activity), and type of enzymes used must be appropriate. Supplementation of diets with carbohydrases and phytases has been shown to enhance the availability of minerals and other nutrients. It is therefore important to make sure that diets containing unavailable nutrients, are designed to account for the increased availability of the nutrients following supplementation with exogenous enzymes. Failure to do so may allow nutrients to be wasted as well as to cause increased water consumption with consequential adverse effects on litter quality and animal health, product quality and the environment. Furthermore small improvements seen with relatively small numbers of animals under experimental conditions can translate into considerable financial savings in producing millions of animals per annum.

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**The effect of auditory stimulation on the behaviour of kennelled dogs**

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**Introduction** The value of auditory enrichment for psychological well-being has been studied in a variety of species, including birds, cattle, horses and primates. To date the effect of auditory stimulation on the behaviour of dogs housed in rescue shelters is unknown. Rescue shelters provide temporary housing for thousands of stray and abandoned dogs every year. However well these dogs are cared for, it cannot be ignored that being in such a situation is stressful. Research suggests that music may be a useful moderator of stress in humans. The question remains as to whether auditory stimulation has such a beneficial effect in dogs. This study investigated the behaviour of sheltered dogs in response to five types of auditory stimulation to determine whether the dogs' behaviour was influenced by their auditory environment.

**Materials and methods** Twenty-seven neutered male, and 23 spayed female, dogs, housed at a National Canine Defence League Rehoming Centre, were used as subjects. Five conditions of auditory stimulation were developed, namely: (1) Control (no auditory stimulation other than that arising naturally from the animals' environment); (2) 'Human Conversation' (radio programme devoted purely to conversation); (3) Classical Music (tracks from 'The Very Best of the Classic Experience'); (4) Heavy Metal Music (tracks from Metallica's 'Metallica' album), and; (5) Pop Music (tracks from the 'Now 44' album). Dogs were exposed to each condition of auditory stimulation for 4 hours on 5 separate days. For each condition, the dog's vocalisation, activity and position in cage, were recorded every 10 minutes for 4 hours.

**Results** The type of auditory stimulation the dogs were exposed to significantly influenced the animals' vocalisation ($P<0.001$). Dogs spent more time quiet and less time barking during the classical music condition, and more time barking, and less time quiet, during the heavy metal condition (Figure 1). The dogs' activity was significantly related to type of auditory stimulation ($P<0.001$). Dogs spent more time resting and less time standing during the classical music condition (Figure 2). The dogs' position in their cages was not significantly related to type of auditory stimulation.

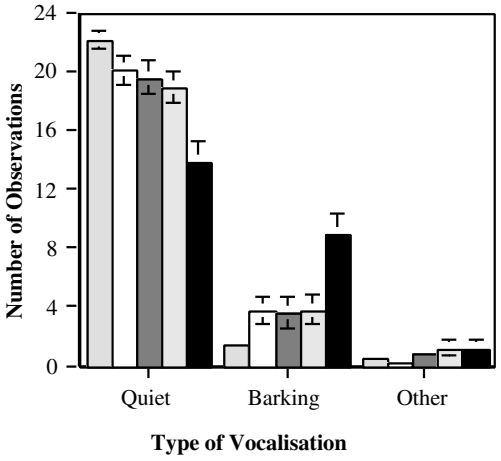


**Figure 1** Effect of music on vocalisation

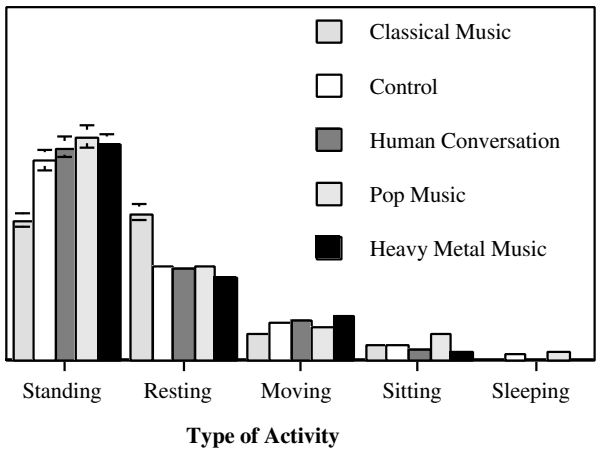


**Figure 2** Effect of music on activity

**Conclusions** The results suggest that the behaviour of sheltered dogs is influenced by auditory stimulation and that certain types of auditory stimulation may be beneficial for the animals' welfare. It is important that an appropriate form of auditory stimulation is employed in shelter environment. Rescue shelters are extremely noisy environments. Adding further auditory stimulation, particularly that which causes increased stress, may do more harm than good and should be avoided. Providing auditory stimulation that has a calming influence, however, may be advantageous, resulting in improved animal welfare, enhanced perceptions of dog desirability and an increase in the number of dogs that are re-homed.

# Why do people bring dogs back to the pound? A questionnaire study in Milan, Italy

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**Introduction** The ASL (Local Sanitary Agency) dog pound in Milan is a sanitary structure where stray dogs (dogs which have run away from their owners, abandoned dogs and free ranging dogs) are housed after being found. Dogs cannot be adopted during the first 10 days. After this period, dogs stay in the same structure if space is available (and can be adopted), otherwise they are put in private kennels for unlimited time. Only dogs which are seriously sick or proved to be aggressive and dangerous can be put down through euthanasia (Law 281/91). When an adopted dog is brought back to the pound, the adoptive owner is asked to fill in a questionnaire. However, not everyone accepts to do so.
The aim of this study is to understand: a) the reasons why dogs adopted from the pound are then left again; b) what influences the owner's decision to leave their pet; c) if the decision is linked to problems caused by the dog or to the owner's choice.

**Material and methods** The study considers the period between January 1998 and September 2001. A total of 3,422 dogs entered the pound; 1,789 dogs were adopted and 271 of them were brought back to the dog pound after adoption. A total of 173 questionnaires are available. The questionnaire presents open-ended questions in which the adoptive owners can provide information about motivation for the adoption, causes for repudiation and criteria followed when choosing that particular dog. The questionnaire also presents multiple choice questions aimed at describing the adoptive family's life style and the dog's behaviour and habits.
First, a descriptive analysis was carried out in order to have a general view of the adoption reality at these dog pound. Data were then re-arranged into categories for statistical analysis. Analyses of variance were carried out on the length of adoption in the new home, excluding dogs kept for less than a week. Different groups of adoptive owners were considered on the basis of motivation for adoption, criteria when choosing the dog, and reasons for repudiation.

**Results** 127 out of 173 repudiated dogs were mixed bred (73.4%), with 111 males and 62 females. 57.2% of these dogs were younger than 1 year, 38.7% were younger than 5, and 4.1% were older than 5 years. Most of the adoptive owners (60%) had had previous experience with dogs, nevertheless they were not able to deal with the problems that they experienced with the adopted dog. In fact, about 40% of the dogs stayed in the new home less than 7 days (mean 2.8) and 60% of them stay longer (maximum 275 days; mean 56.4).
The ANOVAs showed that the mean length of stay in the new home is influenced by the motivation for adoption only: dogs adopted for company were significantly kept longer than those adopted on the basis of a generic "love" for animals ($F=4.05$, $df=1.67$, $P=0.048$; figure 1). The criteria used when choosing the dog (dog's physical features, emotional perception of the dog's temperament, or no criteria) and the reasons for repudiation (personal difficulties or dog's behavioural problems) did not influence the length of adoption (Figure 2). 48% of adopted dogs showed destructive behaviour related to separation anxiety when left at home alone.

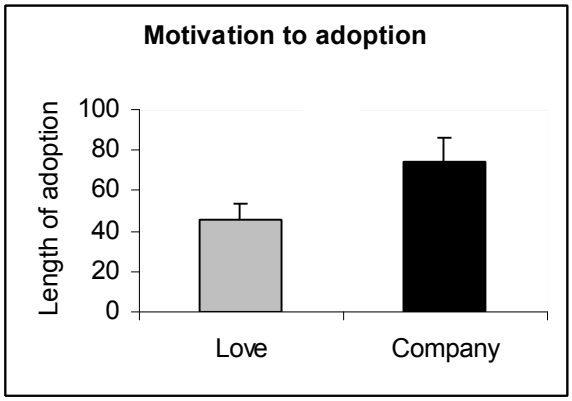


Figure 1

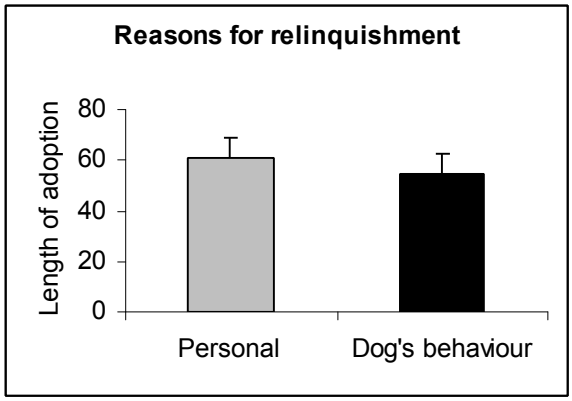


Figure 2

**Conclusions** This study pointed out that 15% of adopted dogs are brought back to the shelter. The motivation for adoption significantly affects the length of adoption. There is no significant difference in the percentage of repudiation due to personal reasons or the dog's behavioural problems. Despite the efforts of the staff at the dog pound over the past few years, the process of adoption is still lacking in the aspects related to the future owner's awareness of the responsibility, effort and care required. It is necessary: 1) to help future owners consider their motivation; 2) to help them in choosing the dog; 3) to follow them up at home in order to solve the dog's behavioural problems.

# Investigating temperament traits in cattle for quantitative trait loci (QTL) identification

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**Introduction** Farm animals show individual variation in their behavioural responses to handling and management systems on farms. These behavioural responses are presumed to reflect underlying temperament traits such as fear or aggression. Information about the location of genes that influence temperament traits could be used in selective breeding programmes to improve animal welfare, as selection for desirable behavioural responses would increase the ability of animals to cope with stressors encountered on farms. The aims of this study were to obtain reliable temperament measurements in cattle using behavioural tests, and to use this data to localise the genes (quantitative trait loci) that are involved in such traits.

Behavioural data obtained in temperament tests must be shown to reflect underlying traits by demonstrating intra-animal repeatability, inter-animal variability and validity. The objectives of this experiment were i) to carry out four behaviour tests on a group of heifers, and examine the repeatability, variability and validity of the results obtained; ii) to correlate the behavioural data with genotyping data from a large number of heifers to look for associations between behavioural phenotypes and genetic markers. Associations localise quantitative trait loci (QTLs), or regions of the genome, that are involved in these traits.

**Materials and Methods** Temperament tests were carried out on 54 12-month-old heifer calves from the second generation cross of a Holstein x Charolais resource herd. Four tests were used; a Flight-from-Feeder test (FF), a Social Separation Test (SS), a Novel Object Test (NO) and a Handling Test (H). The variables measured included locomotory and escape behaviours, and are presumed to reflect underlying traits of fear, aggression and exploratory motivation. Each test was repeated twice per individual to assess repeatability. Restricted Maximum Likelihood (REML) was used to calculate repeatability values, and where appropriate Principle Components Analysis (PCA) was used to group behaviours of a common motivational background.

**Results** The FF Test demonstrated a wide range of scores between individuals (Fig. 1), that had high repeatability (REML; r = 0.56 ±0.12). The SS Test also showed a wide range of behavioural response with a high repeatability of durations of 'fearful' behaviours (r = 0.63 ±0.11; Fig. 2). The NO test showed low variability of response, and a low repeatability of 'duration of contact time' (r = 0.26 ±0.12). The HA Test showed a wide variability of response, and a low repeatability of 'latency to be held in corner of the pen' (r = 0.36 ±0.15). No correlations were found between the different test results, suggesting that different traits were being measured in each.

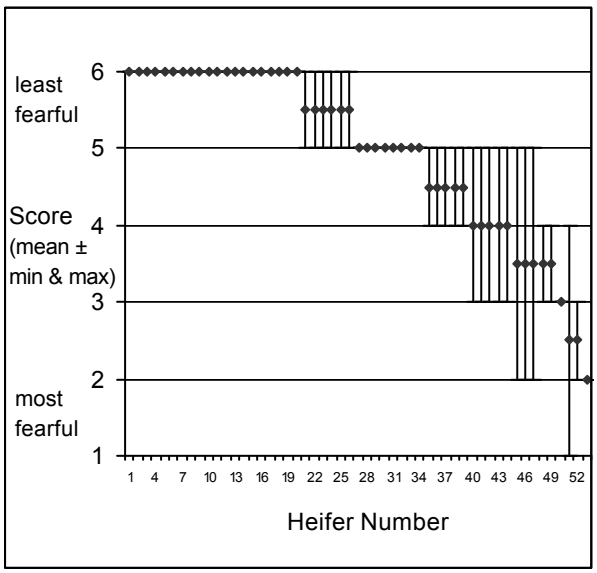


**Figure 1: Flight-from-Feeder Scores.** Score ranges from 1 (heifer moves away when the observer is >2m away) to 6 (heifer doesn't move back when observer touches on the head)

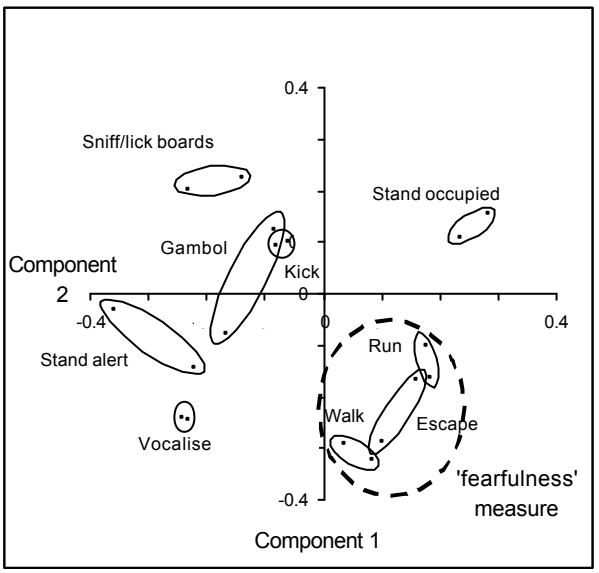


**Figure 2: Principle Components Analysis of behaviours that occur in the Social Separation Test.** Durations of behaviours shown in the 2 tests are ringed. Component 1 explains 26.4% of the variation, Component 2, 11.2%. The summed durations of Escape, Run and Walk are used as a measure of fearfulness (dashed circle).

**Conclusion** The variability and repeatability of the behavioural variables from the FF and SS Tests show that they are suitable for use in potential identification of QTLs. Further validation of the tests will also be presented, along with preliminary QTL analysis carried out using the data from these tests and genotyping data from 200 microsatellite markers.

# The qualitative assessment of pig behaviour using Repertory Grid Technique

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**Introduction** Most studies which provide qualitative assessments of animal behaviour use prefixed lists of adjectives (e.g. shy, bold, interested). However,Wemelsfelder et al. (2000, 2001), using Free-Choice-Profiling (FCP) methodology, allowed observers to develop their own descriptors for scoring pigs, and found this method to be highly reliable and repeatable. Repertory grid technique (RGT) is a frequently used method in human psychology and resembles FCP. Recently RTG has also been applied to assess personality in chimpanzees (Dutton et al., 1997). The purpose of the present study was to apply RGT to pigs and to correlate obtained pig scores with those previously obtained through FCP.

**Materials and methods.** As a basis for this study we used 10 video-clips made for the FCP study by Wemelsfelder et al. (2001), showing 10 individual Large White and Landrace female pigs of about 15 weeks of age. These pigs had been housed together in a straw-bedded pen of 4x3m, and had been trained to stay singly in an identical, directly adjacent test pen for 7 minutes. In this test pen the pigs could interact with a human interactor located in the centre of the pen. RGT was used to instruct seven raters to assess these 10 pigs. RGT has two stages: The elicitation of adjectives from raters, and the scoring of animals. In the first stage the raters watched a 1-minute video clip of each pig, and were instructed by the experimenter to generate bipolar constructs (e.g. "shy-bold") for describing the pigs. The experimenter then composed a common scoring list which retained only those constructs used by the majority of raters. In the second stage raters watched a 4-minute video clip of each pig, and scored the pigs on each term of the common scoring list on a 5-point scale. The degree of agreement between raters was determined by calculating the Kendall coefficient of concordance (W).

**Results.** The results indicate that raters showed highly significant agreement in their scoring of all but one of the pigs ($P<0.001$). Figure 1 shows the spatial configuration of the pigs as determined through Multidimensional scaling. The two dimensions obtained were 1. "shyness – boldness" and 2. "gentleness – roughness". There dimensions and the position of the pigs on them were significantly correlated with the pig scores obtained in the FCP study by Wemelsfelder et al. (1: $r^2 = 0.891$, $P<0.001$; 2: $r^2 = -0.661$, $P<0.05$).

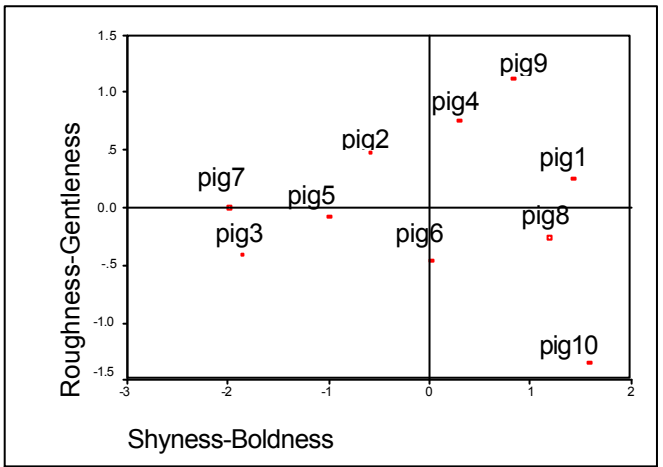


**Figure 1**: Euclidian distance model of configuration for 10 pigs scored on 26 constructs

**Conclusions** There results indicate that raters could systematically use generated constructs as measurement tools, suggesting that RGT is a reliable method for the qualitative assessment of pig behaviour. Furthermore the validity of this method is apparent from a cross-validation between RGT and FCP. Even though different observers were used and pigs were shown in different order, these techniques identified the same dimensions of pig behaviour. We suggest that this provides further support that the qualitative assessment of animal behaviour is based on systematic empirical observation.

**References**

Dutton D M, Clark R and Dickins D W 1997 Personality in captive chimpanzees: use of a novel rating procedure. *International Journal of Primatology* **18**: 539-552.

Wemelsfelder, F., Hunter, E. A., Mendl, M. T. and Lawrence, A. B. (2000). The spontaneous qualitative assessment of behavioural expressions in pigs: first explorations of a novel methodology for integrative animal welfare measurement. *Applied Animal Behaviour Science* **67**: 193-215.

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# Social learning and facilitation of operant key-pecking by domestic hens

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**Introduction** It has been suggested that feather pecking becomes widespread in groups of birds because it is socially learnt. However, a bird pecking the feathers of another seldom gains obvious extrinsic reinforcement for this behaviour (the feather is not always eaten, or material pecked from the plumage). Two aspects of social learning that have received little attention are whether reinforcement of the bird performing the behaviour (the demonstrator) is required for the watching bird (the observer) to learn, and in addition, whether single or multiple exposures to the behaviour are required. In two experiments we used a model behaviour - operant key pecking - to examine the effects of reinforcement of demonstrators (experiment 1) and the effectiveness of repeated demonstrations (experiment 2) on social learning by domestic hens.

**Materials and methods** The apparatus comprised a two-chamber box constructed with a clear partition between the chambers. The 'demonstration chamber' contained two operant keys and a feeder door that was opened by a computer to give access to food whenever a hen made the correct response. The 'observation chamber' had a clear front but was otherwise featureless. Observers saw one of three demonstrations: a demonstrator pecking 60 times for 20 reinforcements of 5 s access to food (R), a demonstrator pecking 60 times but receiving no reinforcement (NR), or, the feeder door automatically opening 20 times with no demonstrator present (control). In experiment 1, observers were randomly allocated to treatments (R; N=20 observers), (NR; N=20 observers), or (control; N=16 observers) and tested after watching 4 demonstrations. In experiment 2, observers were randomly allocated to treatments (N=12 observers/treatment) but were tested after each of 7 demonstrations.

**Results** In experiment 1 there was a significant effect of treatment on the number of pecks by the observers to the front wall ($F_{2,8}$=53.4; P<0.001), the number of pecks to the apparatus front, i.e. pecks to the front wall plus pecks to the keys ($F_{2,8}$=24.6; P<0.001), the number of registered (by the computer) pecks to the keys regardless of whether to the correct key or not ($F_{2,8}$ =15.2; P<0.001), and the total number of registered and non-registered pecks to the keys ($F_{2,8}$ =10.9; P<0.005). In all cases, NR observers performed the greatest number of pecks, control birds the least, and the R birds an intermediate number. Unexpectedly, the mean number of registered pecks to the correct key by the R birds was significantly less than the NR birds ($F_{1,8}$=10.2; P=0.013), i.e. when demonstrators were reinforced for operant key pecking, the number of registered pecks to the correct key by the observers was less than when the demonstrators were not reinforced. For each of the treatments, the mean discrimination ratios were within 1 SEM of 0.5, i.e. observer pecks were distributed uniformly between the two keys.

In experiment 2, there was a very low rate of pecking by the observer birds precluding analysis of the influence of repeated demonstrations. However, there was a significant effect of treatment on observer pecking behaviour overall. The NR observers directed a significantly ($F_{2,31}$=3.45; P<0.05) greater proportion of total pecks to the operant keys than the R observers, and the C birds an intermediate proportion (X ± SEM: 0.27 $\pm$ 0.08; 0.08 $\pm$ 0.04; 0.13 $\pm$ 0.05 respectively).

**Conclusions** These results confirm previous findings that by watching other hens perform an operant response, the probability of the observing birds subsequently performing this behaviour is increased (Nicol & Pope, 1992). However, the present results indicate this is likely to be social facilitation rather than social learning. Both Experiments 1 & 2 showed that reinforcement of demonstrators was not necessary for social facilitation of pecking by observers. This could explain why feather pecking, in which the pecking hen is not always (obviously) reinforced, can spread so quickly through a flock.

**References**

Nicol, C.J. & Pope, S.J. 1992. Effects of social learning on the acquisition of discriminatory key pecking in hens. *Bulletin of the Psychonomic Society*, **30:** 293-296

# The effect of providing a choice of social environment on performance and behaviour of gestating sows

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**Introduction:** The move towards group housing of gestating sows has highlighted a number of welfare issues associated with social interactions that are directly related to management and pen design. For example feeding method and total space can impact on both behaviour and performance. Individual feeding stalls combined with a straw based lying area and dunging passage have production and behavioural advantages at feeding time by providing safety from aggressive pen mates. Anecdotal observations would suggest that they also provide an alternative lying space. However, in practice many feeders are either kept closed when not being used or are located in areas that do not provide a good thermal environment. This experiment was designed to evaluate environmental and social factors affecting the choice of lying behaviour of sows with and without access to feeding stalls throughout the day.

**Materials and methods:** Eighty-two, parity 2-4, PIC Camborough 15 sows were monitored from two weeks post service through to farrowing. Mean sow weight and backfat at weaning were 223kg (sd 21.0) and 21mm (sd 3.7) respectively. They were allocated to one of two treatments over six replicates, group size varied between 5 and 8 sows. Treatments were: (RA) restricted access to feeding stalls, only at feeding time, and (FA) free access to the feeding stalls at all times. The feeding stalls had a sow operated back gate that provided complete security from other group members. The pens were situated within an insulated, environmentally controlled building. The pen dimensions were the same for all groups and consisted of a strawed lying area (11.54 m²), dunging area (8.15m²), and feeder area (8.71m²). With the range in group size and the restricted access the total space per sow ranged from 2.50m² to 5.75m². This was above the current recommendations and considered to be in the correct range to promote adequate welfare (Weng, *et al*. 1998). The hierarchy of each group was determined in week 12 by a paired food competition test and sows were categorised as top or bottom half. Lesion scores were measured on a scale 0-5 at 2, 6, and 12 weeks into gestation. Sow behaviour was recorded on time-lapse video for 48 hours in week 12, and analysed for location in pen and activity. Whilst video recording, the temperature was data logged in the three areas of the pen. The results were analysed by ANOVA.

**Results:** The total time spent lying was not affected by treatment or hierarchy (Table 1). There was a clear indication that the sows at the bottom of the hierarchy made use of the feeders for lying and where this was restricted they chose to lie in both the dunging and lying area. The air temperatures of the sows' lying location were significantly different 18.6, 18.3 and 17.5°C for the lying, dunging and feeding areas respectively (sed 0.22, P<0.001). There was little evidence that this difference affected lying behaviour. Although the overall levels of lesions was not considered to be particularly high and there were no treatment effects, there was a significant difference between the bottom and the top of the hierarchy. Overall performance was good. Given the range in group size, there was no correlation of space per sow and number born. There was however, a significant effect in number born which can be attributed to the greater choice given to the free access treatment (Table 1). There were no treatment differences in parity or conception.

**Table 1** The effect of restricted access to individual feeders on behaviour and performance of sows

| **Treatment (T)** | **Free access to feeders** | | **Restricted access to feeders** | | | **Significance** | | |
|---|---|---|---|---|---|---|---|---|
| **Position in Hierarchy (H)** | **Top** | **Bottom** | **Top** | **Bottom** | **Sed** | **(T)** | **(H)** | **(TxH)** |
| Time spent lying as a proportion of observations over 24 hours | 0.82 | 0.86 | 0.82 | 0.83 | 0.013 | NS | NS | NS |
| Time spent lying in lying area as a proportion of time spent lying | 0.84 | 0.56 | 0.85 | 0.81 | 0.002 | 0.005 | 0.001 | 0.006 |
| Time spent lying in dunging area as a proportion of time spent lying | 0.02 | 0.10 | 0.15 | 0.18 | 0.028 | <0.001 | 0.052 | NS |
| Time spent lying in feeders as a proportion of time spent lying | 0.14 | 0.34 | 0.00 | 0.01 | 0.062 | | 0.002 | |
| Mean face lesion score over gestation. | 0.74 | 0.77 | 0.56 | 0.80 | 0.071 | NS | 0.053 | NS |
| Mean body lesion score over gestation. | 0.56 | 0.88 | 0.69 | 0.80 | 0.104 | NS | 0.044 | NS |
| Total number of piglets born | 13.95 | 13.47 | 12.53 | 11.28 | 0.814 | 0.049 | NS | NS |

**Conclusions:** In what may be considered a relatively high welfare system giving sows a choice of lying area in gestation can increase sow productivity. The welfare of bottom rank animals may be compromised if they are obliged to share the same space as their high ranking contemporaries.

**Reference:** Weng, R.C., Edwards, S.A. and English, P.R., 1998. Behaviour, social interactions and lesion scores of group housed sows in relation to floor space allowance. *Applied Animal Behavioural Science*, **59**, 307-316.

# Breed differences in the expression of maternal care at parturition persist throughout the lactation period in sheep.

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**Introduction** In the ewe maternal care begins at parturition with grooming of the neonate and cooperation with early sucking attempts (e.g. Shillito-Walser 1978). Subsequently maternal care is expressed through sucking interactions, a close ewe-lamb association and a tendency to seek absent lambs (e.g. Hinch *et al* 1987). The ewe increasingly controls sucking interactions using a 'head-up' posture to signal when the lamb may approach and suck (Lawrence 1984).
Two breeds of sheep, Suffolk and Scottish Blackface, are known to differ in their initial maternal behaviour. Blackface ewes spend longer grooming their lambs, show more cooperation with sucking attempts and less negative behaviour such as aggression and rejection, when compared to Suffolk ewes (Dwyer & Lawrence 1998). The aim of this study was to examine whether these breed differences in the expression of maternal care persist throughout the lactation period.

**Materials and method** The maternal behaviour of 32 primiparous ewes (18 Suffolk, 14 Scottish Blackface) was observed throughout lactation. Ewe behaviour was observed continuously for 30 minutes after parturition. At 3 days post-partum ewes were subject to a maternal choice test between their own and a similar alien lamb. Between 4 and 28 days 2x15 minute focal observations were made of each ewe every second day and 10 scan samples of the whole flock were made each week. Between 5 and 10 weeks focal observations were reduced to 2x15 minutes per week. Data were checked for normality, transformed where possible, and analysed using REML, Chi-square, T-Test or Mann-Whitney accordingly.

**Results** Blackface ewes were quicker to begin grooming and spent longer licking their lambs after parturition when compared to Suffolk ewes (Table 1). In the maternal choice test Blackface ewes were quicker to reach their own lamb, more likely to approach their own lamb first and spent longer with them during the test than Suffolk ewes (Table 1). Lambs with Blackface dams had a higher proportion of sucking attempts accepted by the ewe (Figure 1) at weeks 2 ($U=207.5$, $p<0.01$), 4 ($U=215.0$, $p<0.01$), 6 ($U=142.0$, $p<0.01$) and 8 ($U=156.5$, $p<0.01$). Blackface ewes were closer to their lambs throughout lactation (Suffolk=28.72m, s.e.m.=3.303, Blackface=16.16m, s.e.m.=1.36, Wald=9.0, 1 d.f. $p<0.01$) and also showed a higher frequency of 'head-up' postures (Suffolk=0.345/observation s.e.m.=0.052, Blackface=0.737/observation, s.e.m.= 0.12, Wald=11.5, 1 d.f. $p<0.01$).

**Table 1** Parturition data and maternal choice test.

| | Suffolk | Blackface | |
|---|---|---|---|
| Latency to groom (s) | 287.1 (s.e.m.=87.3) | 173 (s.e.m.=126) | Wald=5.7, 1 d.f. $p<0.05$ |
| Duration of grooming (s) | 519.0 (s.e.m.=82.3) | 1110 (s.e.m.=138) | Wald=9.5, 1 d.f. $p<0.01$ |
| Time to reach own lamb (s) | 6 (Q1=3.5, Q3=18.5) | 4 (Q1=1.75, Q3=5) | U=326.0, $p<0.05$ |
| Approach own lamb first | 53% | 90% | $\chi^2= 9.06$ 1 d.f. $p<0.001$ |
| No. scans spent with own lamb | 8.24 (s.e.m.=1.2) | 15.43 (s.e.m.=1.6) | T=-3.56, 25 d.f. $p<0.01$ |

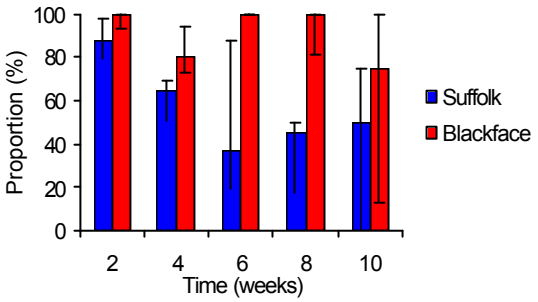


**Figure 1** Proportion of sucking attempts accepted by the ewe (medians, Q1, Q3 shown.).

**Conclusions** The results indicate that breed differences in maternal care do persist throughout the lactation period. Blackface ewes have a greater tendency to groom their lambs after parturition, show a greater attraction to their lambs at 3 days post-partum and are closer to them throughout the lactation period, compared to Suffolk ewes. They show more communication with their lambs via the 'head-up' posture and their lambs have a higher proportion of successful sucking attempts. This suggests that Blackface ewes have a closer association with their lambs compared to Suffolk ewes. A close ewe-lamb association is likely to be advantageous in protecting the lamb from predators, preventing it from becoming separated from the flock and consequently improving lamb survival.

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Shillito-Walser E.E., 1978. The development of the maternal-offspring bond in sheep. *Applied Animal Ethology* **4:** 293-294.

# Individual differences in sociability and the trade-offs made by sheep grazing in a patchy environment

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**Introduction** In a patchy environment, sheep may have to make trade-offs between being close to companions and grazing the preferred vegetation. It has been demonstrated that individual differences in sociability, measured as the tendency to graze close to others in a group, can predict behaviour in a motivational conflict situation (Sibbald *et al*, 2000). An experiment was carried out, in which sheep with different sociability indices were compared in a test situation which required single animals to move away from the group in order to graze, but where stress due to physical separation was minimised.

**Materials and Methods** In order to select animals with high and low sociability indices, 4 groups of 11 one-year-old, female, Scottish Blackface sheep were each observed while grazing in a 30 x 33 m grass plot, for periods of one hour, on 10 independent occasions. Sociability indices (Sibbald *et al*., 1998) were calculated from the frequencies with which focal sheep were nearest neighbours to other sheep in the group. Two individuals from the top of the range of sociability indices ("sociable") and two from the bottom ("unsociable"), were selected from each group. The 16 selected sheep were then tested individually, by separating each from its group and moving it to a test area on the other side of a simple wire-mesh fence. At no time were the single sheep forced to move more than a few metres away from their companions. Each test area comprised a strip of ground 16 x 66 m, with an area of long grass beginning either 0, 15 or 35m away from the dividing fence. The grass between the fence and the long grass was cut as close to the ground as possible immediately before behavioural tests were carried out, in order to minimise any grazing on these areas. Tests were carried out on 2 sociable and 2 unsociable sheep per day, on a total of 12 test days. All sheep were tested with the 0m treatment first and the subsequent order of presentation of the 15 and 35m treatments was balanced within both the sociable and unsociable sheep. Each test lasted 75 minutes, during which behaviour and distance from the fence were recorded every 30 seconds. Bite rates were also measured by counting the total number of bites taken over a 2-min period every 15 mins throughout the test.

**Results** Each individual in a group will have a sociability index of 1.0 if they are all observed to be another sheep's nearest neighbour the same number of times, with higher or lower values indicating sheep that are more or less sociable than each other. The mean sociability indices for the 8 sociable and 8 unsociable sheep selected for the tests were respectively 1.2 and 0.83 (SED 0.049). For all sheep there was a significant effect of treatment on mean distance from the fence during the tests (18.0, 24.8 and 31.8 m, when long grass was 0, 15 and 35m away). Sociable sheep tended to be nearer to the fence than unsociable sheep (21.0 v 28.7 m, SED 5.32) and to spend less time on the long grass during the test (49.3 v 60.7 min, SED 10.49). Mean bite rates and estimated total bites taken during the test were both significantly lower in sociable than unsociable sheep when the long grass was 15m away (35.9 v 51.1 bites per min, $p=0.009$; 2378 v 3621 bites, $p=0.012$), but there were no differences at 35m. When the long grass was 35 m away, individual variation within both sociable and unsociable animals was high; some individuals stayed close to the fence, some grazed throughout and others alternated between grazing and returning to the fence to be beside their companions.

**Conclusions** Distances between grazing sheep are likely to reflect a number of processes. The sociability index depends not only on the tendency of the focal sheep to graze close to others in the group, but also on the tendency of the other animals to graze close to the focal sheep. In addition, sociability may result from a fear of being alone or from attraction to a particular companion or the motivation to seek companionship in general. Sociability is measured when sheep are grazing on a uniform sward, when the spatial distribution of the vegetation should not influence the sheep's movements. When making a trade-off between staying with the group or moving away to graze in a patchy environment, the strength of the motivation to feed will become an important factor. The results of this experiment demonstrate that while the sociability index can predict behaviour while sheep are operating within the range of nearest neighbour distances that is characteristic for the breed, when individuals have to move further away in order to graze, other factors come into play.

**Acknowledgements** This study was funded by the Scottish Executive Rural Affairs Department.

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# Spatial distribution during grazing reflects dominance relationships between individual sheep

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**Introduction** Female sheep do not frequently show overt dominance displays while grazing on pasture. This has led to the conclusion that, for female sheep, dominance at pasture is of little importance. While sheep show few agonistic interactions, they do make clear and consistent choices of who they graze close to. This experiment was designed to answer the question whether these choices reflect the underlying dominance relationships between individuals, potentially being the reason for the low level of agonistic interactions on pasture.

**Material and methods** The project consisted of two parts, carried out in summer and autumn of the same year:
Part I (June/July; indoors, competition) - 10 one-year-old female Scottish Blackface sheep were housed indoors in a group pen, where they had free access to food twice daily, from 0700 to 0800 h and from 1900 to 2000 h. They were tested in pairs in a separate test arena for 5 minutes at 0800, 1000, 1200, 1400 and 1600 h. Each possible pair was tested once at each of these times, but never twice on the same day. A feed-hopper restricted access to food to only one sheep at a time. We recorded the number of displacements, non-aggressive (by nudges) as well as aggressive ones (by threats and butts), the time spent feeding or blocking (preventing the other sheep from feeding), and the total food intake. The sheep performing blocking behaviour was considered the dominant partner in the pair. The data from the 1600 h tests were used for this analysis, because they showed the behaviour at the highest level of feeding motivation.
Part II (September; outdoors, at pasture, sociability) - We recorded the identity of each sheep's nearest neighbour during grazing on 10 separate one-hour-sessions (scan samples at 4 min intervals) and calculated the relative frequency with which the individuals in each possible pair were each other's nearest neighbour. We then chose the ten pairs with the highest (most sociable pairs) and the ten with the lowest frequency of association (least sociable pairs), and used them for the analysis of the data from part I.

**Results** Competition: When blocking behaviour was only shown in a competition test, it was only performed by one sheep of the pair, providing clear information on dominance status. It was therefore used to categorise the sheep into dominant and subordinate. The total number of displacements in a competition test ranged from 0 to 123, the food intake per pair from 220g to 680g. Of the total time spent feeding, dominant sheep spent more time feeding than subordinate ones (65.9% ± 5.14 v 16.7% ± 3.83; paired t-test, n= 20, T=5.61, $p<0.001$) and they performed more aggressive displacements (4.25 ± 1.58 v 0.5 ± 0.28 for dominant and by subordinate sheep, respectively; paired t-test, n= 20, T=2.41, p=0.02). Dominant sheep used both aggressive and non-aggressive displacements, subordinate sheep used almost exclusively non-aggressive displacements (non-aggressive displacements for dominant and subordinate sheep: 63.3% ± 8.58 v 96.9% ± 1.83; paired t-test, n= 13, T=3.57, p=0.004). In 10 pairs, the subordinate sheep spent hardly any time at the feeder (0 to 2.8 %, 'non-sharing'), in the other 10, the subordinate spent between 23.7 and 56.4% of the time at the feeder ('sharing'). Total food intake for a pair increased with the amount of sharing ($r_P$ = 0.70, $p<0.001$). The dominant partner did not get less food when sharing with the subordinate (food intake of the dominant sheep, estimated from proportion of time at the feeder x total food intake of the pair, for those who shared and those who did not: 330.7g ±18 v 351.0g ±36, t-test, T=0.52, df=13, p=0.62).
Sociability: The ten least sociable pairs were recorded as each other's nearest neighbour on 5.2 % ± 0.52 of the occasions (range: 2.8% to 6.9%) and the ten most sociable pairs on 19.2% ± 1.78 of the occasions (range: 13.4% to 31.3%).
Links between competition and sociability: There were more displacements in most than in least sociable pairs (48.9 ± 12 v 11.4 ± 5.5, t-test, df=12, p=0.017), but they did not result in a difference in the total time each pair spent feeding during the competition test (250.9sec ± 11.0 v 244.9sec ± 8.3, t-test, df=16, T= 0.43, N.S.). The subordinate partners in the least sociable pairs performed fewer displacements than those in the most sociable pairs (5.4 ± 2.8 versus 23.6 ± 6.1; t-test, df=12, p=0.019), and therefore spent less time at the feeder than those in the most sociable pairs (proportion of total feeding time spent feeding by the subordinate sheep of the least and most sociable pairs, respectively: 7.4 ± 4.1 % and 35.3 ± 6.40 %, t-test, df=15, T=3.66, p=0.002).

**Discussion** Pairwise competition for food resulted in clear differences between pairs. In some pairs, the subordinate partner did not displace the dominant one and spent no or only very little time at the feeder. This is unlikely to be a result of a low motivation to feed, since in other pairs the subordinate partner spent between a quarter and half of the total feeding time at the feeder. It is more likely to be a result of reluctance by the subordinate to challenge the dominate partner. The dominant partners did not appear to get less food when sharing, which suggests that this behaviour was not a direct result of feeding motivation, but rather a consequence of the personal relationship between the two individuals. Dominance was shown not to be an all-or-nothing phenomenon, where being subordinate means getting no food at all, but rather a relative one, where a subordinate can get as much as half of the food, depending on who the dominant partner is. Since all these aspects of behaviour in the competition test were related to the frequency with which the pairs were nearest neighbours in the field, we conclude that the spatial distribution of sheep when they are grazing reflects their underlying relationships: not so much which partner is dominant, but how the two sheep treat one another.

**Acknowledgements** This study was funded by the Scottish Executive Environment and Rural Affairs Department.

# Meta-analysis of experiments investigating cadmium accumulation in the liver and kidney of sheep

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**Introduction** Cadmium (Cd) accumulates in the human food chain and poses a risk of kidney dysfunction (Fanconi Syndrome) and bone disorders in humans. The margin of safety between typical Cd intakes by humans and levels associated with toxicity is smaller than for other metals. Consumption of just one sheep kidney could cause an average adult person to exceed their Provisional Maximum Tolerable Daily Intake. However, the rate of accumulation in sheep's liver and kidney, the primary target organs for Cd accumulation, is unclear. This makes prediction of the effects of varying Cd intake by sheep on the Cd concentration in these organs difficult. We undertook a meta-analysis of independent feeding trials, which sought to integrate previous findings in order to review existing legislation on permitted levels of Cd in animal feeds and organs. Resulting predictions on Cd accumulation in sheep liver and kidneys are applicable to the broad set of exposure situations investigated in the individual studies.

**Materials and methods** From an initial database of 3800 articles on various aspects of cadmium and ruminants, 21 feeding trials involving 554 animals in a total of 90 treatment and control groups were identified for inclusion in the meta-regression. Estimation of the parameters (feed Cd concentration, dry matter intake, experiment duration, Cd species and delivery method, animal age, weight and gender) was performed using a random effects model due to the high degree of heterogeneity of the data and the models' later predictive use. A step-down procedure removed the most non-significant term ($P>0.05$) from the model, which was then re-run until only significant terms ($P<0.05$) were left in the model. Estimation was performed by Gibbs sampling, using the BUGS software package (Bayesian statistics). The studies were weighted according to methods typical for Random Effects Modelling, incorporating both within and between trial variability components. Conversion factors were derived from the literature to convert liver and kidney wet weight to dry weight concentrations in some studies.

**Results** For sheep, the two major predictors of Cd concentration in liver and kidney were the duration of exposure to Cd (in days) $d$ and the Cd concentration in the feed (in mg/kg DM) $p$. The product of the two, $pd$, however, was a better predictor than the separate or additive evaluation of the parameters within the framework of the model. The only other significant ($P<0.05$) factor which increased liver and kidney Cd concentration in sheep was the presentation of organically rather than inorganically bound Cd. Other variables investigated were rejected by the model as their influence on accumulation was not significant ($P>0.05$).

For sheep kidney: Cd concentration (mg/kg DW) $= 0.36 + 0.030 * pd - 5.42$; if feed Cd is inorganically bound
Cd concentration (mg/kg DW) $= 0.36 + 0.030 * pd$; if feed Cd is organically bound
For sheep liver: Cd concentration (mg/kg DW) $= 0.44 + 0.0064 * pd - 3.64$; if feed Cd is inorganically bound
Cd concentration (mg/kg DW) $= 0.44 + 0.0064 * pd$; if feed Cd is organically bound

Current maximum permitted levels of feed Cd were entered into the sheep model to show the extent to which the model predicts that tissues will exceed fitness for human consumption (under current EU and UK legislation). This model predicted that the kidneys of sheep consuming organically-bound Cd at the highest permitted concentration in food (1 mg/kg feed at 12 % moisture) would exceed the maximum permitted Cd concentration (1 mg/kg wet weight) after a mean of 130 days. The corresponding limit for Cd in liver is 0.5 mg/kg wet weight, which according to the model would be reached after 175 days of consuming organically-bound Cd included in the diet at the maximum concentration. Corresponding values for sheep offered feed containing inorganically-bound Cd are 291 and 674 days for kidney and liver, respectively.

**Discussion and conclusions** Most Cd consumed by sheep is in the organic form (bound to plant protein), so the values derived by experiments using inorganically-bound Cd are less relevant. The maximum time that sheep can be offered feed containing the legally permitted maximum concentration of Cd before their organs exceed maximum permitted Cd concentrations is therefore 130 days. A sheep's lifespan can easily exceed 130 days after weaning, and it is therefore not surprising that sheep organs routinely exceed Cd limits. Potential measures to prevent increased risk to human health from dietary Cd of animal origin include preventing the livers and kidneys of older animals entering the human food chain and strict implementation of current legislation. Reducing the animal's duration of exposure to high Cd feed would be an expensive procedure. Lower limits on Cd concentration in sheep feed would enable the offal of older animals to be included but would be difficult to impose, as herbage that has been regularly fertilized with phosphorus or treated with sewage sludge already sometimes exceeds the legal limit. Routine removal of the liver and kidney of at least mature ewes from the human food chain is desirable in order to reduce human Cd intake.

**Acknowledgements** Royal College of Veterinary Surgeons John Lord Perry Scholarship for S. Prankel. This study was supported by the EU Copernicus programme (Project No. IC15-CT97-1003).

# The metabolizable protein requirement of the parasitized, lactating ewe

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**Introduction** It has been suggested that periparturient breakdown of immunity to parasites (BIP) has a nutritional basis (Coop and Kyriazakis, 1999). In support of this hypothesis, recent studies have shown that an increased supply of metabolizable protein (MP) reduces the magnitude of periparturient BIP in sheep (Houdijk et al., 2001). Improved MP supply to ewes, infected with gastrointestinal nematodes, has been associated with reduced nematode egg excretion and smaller worm burdens. These immunity indicators may differ in their sensitivity to changes in MP supply. This hypothesis has been addressed in the current doses-response experiment, which also allowed us to estimate the MP requirements of parasitized, lactating, twin-rearing ewes. The latter can be expected to be larger than those of non-parasitized ewes, due to MP requirements for mounting an immune response and for replenishing protein losses.

**Materials and Methods** Thirty twin-bearing Greyface ewes were trickle infected with 10,000 infective *Teladorsagia circumcincta* larvae per day, three days per week, from $day_{-46}$ onwards (relative to expected parturition). Daily food allowances during pregnancy were calculated to provide 0.80 times MP requirements. Daily food allowances during the first four weeks of lactation were calculated to provide 0.65, 0.80, 0.95, 1.10 or 1.25 times MP requirements (n=6). The latter was estimated to average 295 g MP per day for non-parasitized ewes (AFRC, 1993). All daily allowances were calculated to provide 0.90 times the metabolizable energy requirement (AFRC, 1993). Ewes and lambs were weighed regularly, and faecal egg counts (FEC, in eggs per gram fresh faeces, epg) were assessed twice weekly from $day_{-37}$ onwards. Ewes were slaughtered at $day_{28}$ to assess worm burdens. FEC and worm burdens were log-transformed; means are reported as backtransformed means with 95% confidence intervals. ANOVA was used to assess the effect of MP supply on BW-loss, calculated milk production (from lamb weight gain) and worm burden. FEC during lactation was analyzed using repeated measures, with FEC during pregnancy as covariate.

**Results** Mean ewe and litter weight at lambing was 67.7±1.09 and 9.3±0.25 kg, respectively. BW-loss of the ewes decreased with an increasing MP supply ($P<0.05$). Figure 1 shows the mean milk production and FEC during lactation, as well as the worm burden measured at the end of lactation ($day_{28}$). MP supply affected calculated milk production ($P<0.01$). Repeated measures analyses showed that MP supply significantly affected FEC during lactation ($P<0.05$), with a significant contribution of the covariate ($P<0.01$) but there was no interaction between MP supply and time ($P>0.20$). MP supply affected worm burden, though this was less pronounced than its effect on FEC ($P=0.054$).

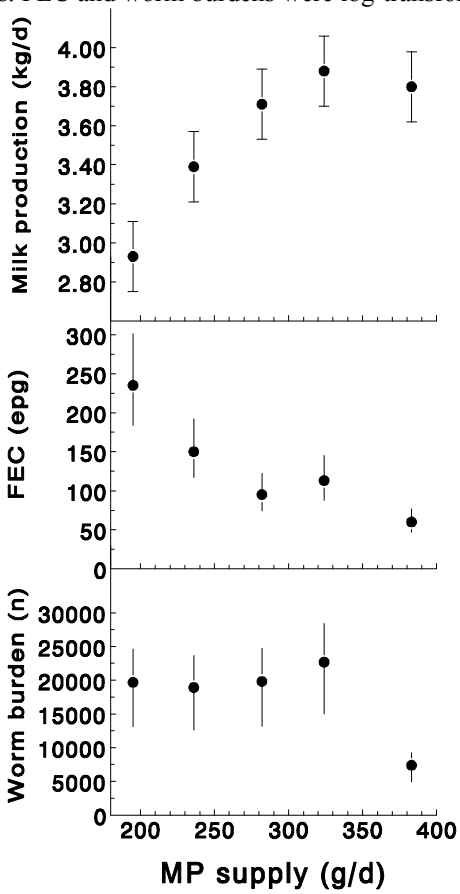


**Figure 1.** Milk production, FEC and worm burden of twin-rearing ewes, offered different allowances of MP

**Conclusion** This study provided evidence that the magnitude of periparturient BIP is sensitive to MP supply. However, the data indicated that immune responses that regulate the size of the worm burden may be less sensitive towards changes in MP supply than those regulating their fecundity. Furthermore, the milk production data suggests that MP requirements of the parasitized, twin-rearing Greyface ewes was ~280 g MP per day, suggesting that MP requirements for non-parasitized ewes (AFRC, 1993) may be overestimated.

Overall, the study supports the view that improved protein supply to ewes could enhance immunity to gastrointestinal nematodes and thus reduce our dependency on anthelmintics for gastrointestinal nematode control.

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

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# The effect of rearing regime on the development of the mammary gland and claw abnormalities in high genetic merit Holstein-Friesian dairy herd replacements

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**Introduction** Feeding and management during the rearing period has a major effect on the subsequent performance and welfare of dairy herd replacements. Recently, Carson *et al*. (2000) found that increasing the live weight of high genetic merit Holstein Friesian heifers from 540 to 620 kg at first calving increased first lactation milk yield by 11%. Mammary growth is a major determinant of milk yield capacity and longevity of lactation and may be the primary driver behind the observed increases in milk production with larger heifers. The first objective of the current study was to evaluate the effects of rearing regime, in terms of diet offered and target weight at 18 months of age, on mammary gland development of high genetic merit Holstein-Friesian heifers. The second objective of this study was to investigate the effect of rearing regime on solear haemorrhages and heel erosions in Holstein-Friesian heifers.

**Material and methods** One hundred and eight high genetic merit Holstein Friesian heifers (PTA (95) for fat and protein yield 48.7 (s.d. 7.26) kg) were used in this study. Heifers commenced the study at 7 weeks of age when they were allocated to four rearing treatments, balanced for source of animal, live weight and genetic merit. Target live weights at 18 months of age were 420 kg (treatment 1) and 466 kg (treatments 2, 3 and 4). Treatment 1 heifers were offered grass silage-based diets during the winter periods and grass-based diets during the summer. Treatment 2 heifers were offered the same forage base along with additional concentrate supplementation. Treatment 3 heifers were offered a straw/concentrate diet during the winter periods and a grass-based diet during the summer. Treatment 4 heifers received the same diets as treatment 3, apart from the summer period when they were housed and offered a straw/concentrate diet. The end-point of the trial was at the completion of the second winter period when the heifers were of an average age of 18 (s.d. 0.7) months. Immediately after slaughter the udder of each of the heifers was dissected from the abdominal wall and stored at $-20^0$C prior to tissue dissection and subsequent chemical analyses. Distal limbs were collected, the soles of each claw functionally trimmed, and solear lesion scores and heel erosion scores recorded (Livesey *et al*., 1998). The data were analysed by the Genstat analysis of variance procedure using a randomised block model with source of the animal as the blocking factor. Covariance analysis was used to adjust the data for minor differences in the number of days pregnant between the treatments.

**Results** The weight of dissected udder fat was higher in treatment 2 heifers compared with treatment 1 ($P<0.01$), 3 ($P<0.05$) and 4 ($P<0.01$) (Table 1). The weight of udder parenchyma did not vary significantly between the treatments. However, the weight of parenchymal protein was greater ($P<0.05$) in treatment 3 heifers compared with treatments 1, 2 and 4. In the front lateral claws, lesion scores in the sole area were lower in treatment 3 compared with treatment 4 heifers ($P<0.01$), but did not differ from treatments 1 and 2. Lesion scores did not vary significantly between the treatments in the white line or heel areas. In the front claws, heel erosion scores were higher in treatment 2 heifers compared with the other treatments ($P<0.01$). In the back claws, heel erosion scores were greater in treatment 2 compared with treatments 1 ($P<0.001$) and 3 ($P<0.05$) and greater in treatment 4 ($P<0.05$) compared with treatment 1.

**Table 1.** *The effects of rearing treatment on udder development and claw abnormalities*

| | | Rearing treatment | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | s.e.m. | Sig. |
| Udder fat (g) | | $1210^{a}$ | $1639^{b}$ | $1304^{a}$ | $1256^{a}$ | 95.8 | ** |
| Udder parenchyma (g) | | 1002 | 1007 | 1446 | 1034 | 141.1 | |
| Parenchymal protein (g) | | $88^{a}$ | $76^{a}$ | $140^{b}$ | $89^{a}$ | 17.4 | * |
| Parenchymal lipid (g) | | 400 | 457 | 543 | 423 | 46.7 | |
| Parenchymal DNA (mg/g) | | 0.94 | 0.55 | 0.73 | 0.77 | 0.118 | |
| Heel erosion scores | fore claws | $2.4^{a}$ | $7.7^{b}$ | $3.8^{a}$ | $4.0^{a}$ | 1.24 | * |
| | hind claws | $3.9^{a}$ | $9.7^{c}$ | $6.0^{ab}$ | $7.2^{bc}$ | 1.00 | *** |
| Lesion scores | fore lateral claw | $2.0^{ab}$ | $1.4^{ab}$ | $0.7^{a}$ | $3.2^{b}$ | 0.63 | * |
| (sole area) | fore medial claw | 1.3 | 1.5 | 0.7 | 2.5 | 0.48 | |
| | hind lateral claw | 2.1 | 2.6 | 1.8 | 2.5 | 0.54 | |
| | hind medial claw | 1.3 | 1.1 | 0.6 | 1.4 | 0.45 | |

**Conclusions** Rearing systems for Holstein-Friesian heifers to adopt heavier weights at first calving had no detrimental effects on mammary parenchymal development, but increased the incidence of heel erosions. A straw/concentrate diet during the winter periods had beneficial effects on mammary parenchymal development and reduced the incidence of claw abnormalities compared with grass-silage based diets. Pasture grazing during the summer period compared with maintaining animals indoors on a straw/concentrate also improved mammary development and claw condition.

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# The use of *in vitro* digestibility techniques in determining the nutritive value of barley and barley–based diets for growing pigs

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**Introduction** Information on the digestibility of a feed or feed ingredient is of crucial importance to the feed compounder leading to reduced variability and a more accurate prediction of nutrient content. As it is not possible to carry out *in vivo* studies on every feed or feed ingredient, *in vitro* techniques have been investigated as an alternative means for obtaining this vital information. There have been several methods developed to determine *in vitro* digestion in monogastrics at both the ileal and overall level. It has been suggested that the methods of Boisen and Fernandez (1995; 1997) to determine *in vitro* digestibility of CP at the ileal level and of energy at the overall level, are the most reliable and accurate methods available (Moughan 1999). The objectives of this study were to compare *in vivo* and *in vitro* digestibility coefficients of both barley and barley-based diets and to evaluate the *in vitro* techniques.

**Materials and methods** Barley-based diets were formulated and fed to PVTC cannulated pigs (Van Leeuwen *et al* 1991) from which ileal digestibility of CP and overall digestibility of energy were determined. The diets contained g/kg: barley 650, soyabean meal 283, tallow 30, titanium dioxide 1.5, minerals / vitamins etc. 35.5. *In vitro* digestibility coefficients for the barley (n = 37) and the corresponding barley-based diets (n = 60) were predicted using the techniques developed by Boisen and Fernandez (1995; 1997). Simple regression equations were used to establish the correlation between *in vivo* and *in vitro* coefficients and also to evaluate the repeatability of the techniques. Repeatability was assessed by determining *in vitro* digestibility of barley and diets on two separate days.

**Results** Ileal *in vitro* digestibility of CP for barley ranged from 0.607 to 0.773 with the mean being 0.689. The coefficients for the diets ranged from 0.666 to 0.819 with the mean being 0.739. A poor relationship ($R^2$ = 0.14) was determined between *in vitro* and *in vivo* ileal digestibility of CP coefficients for the diets (Table 1). The mean value for overall *in vitro* digestibility of energy for barley was 0.793 and the range was from 0.684 to 0.818. The coefficients for the diets ranged from 0.715 to 0.829 with the mean being 0.798. The relationship between *in vitro* and *in vivo* overall digestibility of energy was poor ($R^2$ = 0.07) (Table 1). The repeatability of the two *in vitro* techniques was evaluated (Table 2). A strong relationship ($R^2$ = 0.98) was observed between the 2 separate days of analysis for ileal *in vitro* digestibility of CP for the barley. There was a lack of relationship ($R^2$ = 0.07) between the 2 days of analysis for ileal *in vitro* digestibility of CP for the diets. The repeatability of the technique for determining overall *in vitro* digestibility of energy was relatively satisfactory for both the barley and diets (Table 2).

**Table 1** Correlation between *in vitro* and *in vivo* ileal digestibility of CP and overall digestibility of energy

| | *In vitro* | | | *In vivo* | | | |
|---|---|---|---|---|---|---|---|
| | Min | Max | Mean | Min | Max | Mean | $R^2$ |
| Ileal digestibility of CP | 0.765 | 0.856 | 0.820 | 0.666 | 0.819 | 0.739 | 0.14 |
| Overall digestibility of energy | 0.715 | 0.829 | 0.798 | 0.818 | 0.875 | 0.846 | 0.07 |

**Table 2** Repeatability of *in vitro* techniques

| | Day 1 | | | Day 2 | | | |
|---|---|---|---|---|---|---|---|
| | Min | Max | Mean | Min | Max | Mean | $R^2$ |
| Ileal digestibility of CP for barley | 0.611 | 0.769 | 0.710 | 0.609 | 0.759 | 0.699 | 0.98 |
| Ileal digestibility of CP for diets | 0.797 | 0.851 | 0.820 | 0.769 | 0.830 | 0.798 | 0.07 |
| Overall digestibility of energy for barley | 0.704 | 0.814 | 0.774 | 0.688 | 0.803 | 0.768 | 0.49 |
| Overall digestibility of energy for diets | 0.772 | 0.829 | 0.802 | 0.779 | 0.896 | 0.806 | 0.47 |

**Conclusions** The poor relationships obtained between *in vitro* and *in vivo* digestibility coefficients were in contrast to those obtained by Boisen and Fernandez (1995; 1997) ($R^2$ = 0.92 and 0.67 for ileal digestibility of CP and overall digestibility of energy respectively) as were the lower values obtained for *in vitro* overall digestibility of energy compared with the *in vivo* values. The findings from the repeatability study indicate that the values obtained for barley were more repeatable than those obtained for the diets which may be attributable to a lack of uniformity in diet mixing.

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# Digestibility and nitrogen retention in Creole pigs fed with feedstuffs available in peasant systems in south of Mexico

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**Introduction** Peasant pig production in South of Mexico consist in kept pigs in the backyard. This is a complementary activity for the family income. The pigs are a significant source of protein, but their real meaning lay in their role as a "peasants' savings bank", an asset that could easily be tapped into when cash is needed. In this system Creole pigs are used mainly. The Creole pig is descendent from Iberico and Celtico pigs carried from Spain after the conquest of Mexico five hundred years ago. The Creole pig is fatter, have a reduced liveweigth gain and less fertility in comparison to selected breeds of pigs. However, these characteristics are an advantage for the peasant pig system, due to low nutrient requirements of Creole pigs that match very well with the feed available from the agriculture such as maize, Mucuna beans (*Stilozobium deerengianum*) and forages. The objective of this experiment was to evaluate digestibility and nitrogen retention in Creole pigs fed with feedstuffs obtainable from agriculture in peasant systems in comparison with a diet utilised commonly in commercial pig production systems .

**Material and methods** Twelve Creole pigs were used in the experiment. At the commencement of the trial the pigs had a mean liveweight of 39.5 ± 0.8 kg. The pigs were housed in metabolism crates. Due to the number of metabolism creates available, blocks of four pigs were used. The experimental diets utilised in this experiment were as follow: Diet (**A**) corn only (CP 8.2 %). Diet (**B**) 25 % of Mucuna beans (*Stizolobium deeringianum)* previously boiled and dried + 75 % of corn (CP 12.3 %). Diet (**C**) 25 % of guinea grass (*Panicum maximum*) previously sun dried + 75 % of corn (CP 12.5 %) and diet (**D**) soybean + corn, according to recommendations of ARC, (1981) for growing pigs (CP 15.5 %). All diets included vitamins and minerals. This experiment was conducted in two periods, seven days were allowed for diet adaptation and seven days for faeces and urine collection. The pigs were allocated to one of four dietary treatments in a randomised block design with three blocks and one replication per treatment in each block. Data were analysed using the GLM procedure of SAS.

**Results** Results are given in table 1. A lower digestibility of dry matter and NDF ($P<0.05$) was observed in diet C in comparison to diet A, B and D. This effect was related to the fibrous characteristics of diet C. The absorption of nitrogen was lower in diet A and C ($P<0.05$) than in diet B and D. The excretion of nitrogen in urine was higher ($P<0.05$) in diet D compared with diet A, B and C. However, there was no statistical differences in nitrogen retention ($P>0.05$).

**Table 1** Digestibility and nitrogen balance in Creole pigs fed with diets prepared with feedstuffs available in peasants systems in south of Mexico.

| | Diet | | | | |
|---|---|---|---|---|---|
| Item | A | B | C | D | SEM |
| Dry matter intake (kg/day) | 2.2a | 2.2a | 1.5a | 1.9a | 0.10 |
| Digestibility of dry matter (%) | 90.2a | 88.7a | 72.1b | 89.7a | 1.10 |
| Digestibility of NDF (%) | 47.8ab | 54.2a | 0.0c | 30.5b | 2.00 |
| Nitrogen intake (g/day) | 29.0a | 43.4a | 30.0a | 47.2a | 2.03 |
| Nitrogen absorbed (g/day) | 23.7b | 33.2ab | 22.2b | 41.5a | 1.48 |
| Nitrogen in urine (g/day) | 15.1b | 17.8b | 12.3b | 33.5a | 1.24 |
| N retained (g/day) | 8.5a | 15.4a | 9.8a | 7.9a | 1.00 |

Means in the same row with a common superscript are not significantly different ($P>0.05$)

**Conclusions** These results suggests that utilisation of feedstuffs obtainable in peasants systems in south of Mexico to fed Creole pigs are sufficient to allow an adequate nitrogen retention. There was no benefit in use a diet rich in protein as diet D to fed Creole pigs. Utilisation of diets with high levels of protein increased the losses of nitrogen and could increase the cost of feeding.

**Acknowledgements** This work was funded by DFID.

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# The effect of form and placement of feed for newly weaned piglets on growth performance for three weeks postweaning

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**Introduction** At weaning, piglets must adapt to considerable changes in their environmental, immunological, and nutritional status. This period of adaptation is accompanied by a reduction in piglet growth rate that has been associated with the shift from sow's milk to a solid dry diet. Consequently, feeding management strategies that result in increased feed intake may increase piglet growth rate postweaning. This study evaluated the effects of providing feed as a gruel and feeding on floor mats on piglet performance for three weeks after weaning

**Materials and Methods** The experiment was carried out in a commercial wean-to-finish building and used a randomized complete block design with a 2 H 2 factorial arrangement with treatments being: 1) feed form (dry-pellet vs gruel [1:1 water to pellet ratio]) and 2) feed placement (feeder trough only vs floor mat and feeder trough). Treatments were applied for the first 4 days postweaning with feed being delivered four times per day at 0600, 1000, 1400, and 1800 h according to treatment. In addition, pigs had ad libitum access to a pelleted starter diet and to water throughout the study. Pigs (n=864) were allotted at weaning (4.9 ± 0.02 kg BW; 17 ∀ 2 days of age) to pens of 27 animals on the basis of sex (equal ratio of barrows to gilts) and weight. Floor, feeder-trough, and mat spaces were 0.64 $m^2$, 2.26 cm, and 0.05 $m^2$ per pig, respectively. Pig BW was taken at the start, end of wk 1, and end of wk 3, and feed disappearance was measured at end of wk 1 and 3.

**Results** There was a significant ($P < 0.05$) interaction between feed form and feeding location for gain:feed ratio during wk 1 (Table 1). For pigs fed on the mat and at the trough, there was no effect of feed form on gain:feed ratio (0.387 vs 0.371 ± 0.0273 for the dry and gruel, respectively); in contrast, for pigs fed only at the trough, gain:feed was substantially greater with the dry compared to the gruel (0.622 vs 0.395 ± 0.0273). Gruel feeding significantly ($P < 0.05$) increased feed disappearance in both wk 1 (216 vs 180 ± 8.4 g) and wk 1 to 3 (328 vs 289 ± 10.8 g). However, there was no effect of gruel feeding on growth rate ($P > 0.05$) and there was a trend (P = 0.10) for reduced gain:feed ratio for gruel fed pigs (0.595 vs 0.660 ± 0.0226) from wk 1 to 3. Pigs fed on the mat and in the trough compared to in the trough only had greater feed disappearance during wk 1 (246 vs 150 ± 8.4 g; $P < 0.001$) and for wk 1 to 3 (331 vs 286 ± 10.8 g; $P < 0.05$), increased growth rate (92 vs 76 ± 4.6 g; $P < 0.05$) in wk 1, and tended ($P = 0.06$) to have higher growth rate (197 vs 186 ± 3.3 g) for the 3-wk study period. However, gain:feed ratio for the overall study period was not affected ($P > 0.05$) by location of feed.

Table 1 Effect of feed form and placement on piglet BW and growth rates during the 3 wk period

| Item | Feed form | | Feed placement | | s.e.m. |
|---|---|---|---|---|---|
| | Dry | Gruel | Floor mat and feeder trough | Feeder trough only | |
| No. groups | 8 | 8 | 8 | 8 | |
| Live Weight, kg | | | | | |
| Start | 4.9 | 4.9 | 4.9 | 4.9 | 0.02 |
| Wk 1 | 5.6 | 5.5 | 5.6 | 5.5 | 0.04 |
| Wk 3 | 9.0 | 9.0 | $9.2^b$ | $8.8^a$ | 0.07 |
| Daily gain, g | | | | | |
| Wk 1 | 86 | 82 | $92^b$ | $76^a$ | 4.6 |
| Wk 1-3 | 190 | 193 | 197 | 186 | 3.3 |
| Daily feed intake, g | | | | | |
| Wk 1 | $180^a$ | $216^b$ | $246^b$ | $150^a$ | 8.4 |
| Wk 1-3 | $289^a$ | $328^b$ | $331^b$ | $286^a$ | 10.8 |
| Gain:feed | | | | | |
| Wk 1 | - | - | - | - | 0.0273 |
| Dry | | | $0.387^c$ | $0.622^d$ | |
| Gruel | | | $0.371^c$ | $0.395^c$ | |
| Wk 1-3 | 0.660 | 0.595 | 0.601 | 0.654 | 0.0226 |

a, b means within treatment differ (p<0.05)
c, d interaction means differ (p<0.05)

**Conclusions** These results suggest piglets at weaning provided access to feed on floor mats had increased growth rate in the first three weeks postweaning, however, gruel feeding provided no benefit in terms of growth rate.

# Influence of dietary zinc oxide and antibiotic addition on gut morphology, microflora and digesta pH in piglets

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**Introduction** Evidence from studies involving zinc supplementation is equivocal. Carlson *et al*.,(1999) stated that both early- and traditionally weaned pigs must be fed 3,000 ppm Zn for at least the first 2 weeks after weaning to enhance growth. Others have also demonstrated that weanling pigs exhibit increased growth performance when fed pharmacoloigcal concentrations of zinc (Zn) as zinc oxide (ZnO) (e.g. Hahn and Baker, 1993). However, the mode of action has not yet been fully elucidated. Specifically, there is no research available on the effects of pharmacological Zn concentrations on piglet gut morphology and gut microflora load. Accordingly a preliminary study was designed to examine the effects of dietary Zn and growth-promotant levels of avilamycin supplementation on gut morphological characteristic (villus height, width and crypt depth), together with gut microlflora characteristics, and digesta pH which is an independent factor influencing microflora colonisation.

**Materials and Methods** Thirty-six piglets weaned at 25 days of age were individually housed and fed *ad-libitum* a conventional diet based on commercial raw materials meeting energy and nutrient requirements of the weaned piglet. The control group (n=8) received this diet (diet 1). A further 3 groups were fed supplemental Zn as ZnO (2500 ppm; diet 2); avilamycin (40 ppm; diet 3) or a combiantion of Zn & avilamycin (diet 4). The experimental period was for 14 days and 2 piglets from each group were slaughtered on d 2, 4, 6 & 14 with 4 baseline piglets being slaughtered on d 0. Faecal swabs were also taken on d 0, 3, 5, 7, 9, 11 & 13 for microbiological enumeration for *E. coli, coliform* and *lactobacilli* species. At slaughter, digesta samples from the duodenum, jejunum, ileum, colon and rectum were removed immediately and *E. coli, coliform* and *lactobacilli* enumeration undertaken. Furthermore, of ileal samples were analysed for pH. A peripheral blood sample, together with liver and kidney samples were taken for subsequent mineral analysis. In addition, 6-8 cm length pieces of small intestinal tissue were ligated and subsequently fixed and embedded in paraffin wax. From each of these, sections were cut, mounted on slides, stained and examined under a light microscope. Measurements of villus height, width and crypt depth were taken.

**Results** Analysis of data proceeded through establishing linear and non-linear contrasts with time. The number of coliforms decreased (P=0.004) whilst lactobacilli increased (P=0.002) in the digesta samples over time. There was no significant effect of dietary treatment or time on digesta pH. There was however a significant Day x Diet (P=0.031) interaction for daily feed intake with piglets on the Zn (Diet 2) and avilamycin (Diet 3) supplemented diets consuming the most food. FCR also followed a similar pattern; Day x Diet (P=0.059; polynomial 2), with piglets receiving the Zn supplemented diet (Diet 2) displaying better FCR. The effect of treatment on DLWG was significant (P = <0.001) and individual feed intake values were variable. Villus height increased with time (P=0.002) and there was no reduction in villus height which is often characterstic post-weaning (see table 1).

**Table 1:** Effect of dietary treatment on mean villus height (μm)

| | Diet | | | | | ANOVA | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Day** | 1 | 2 | 3 | 4 | **Mean** | **Diet** | **P** | **Day** | **P** |
| 2 | 388 | 336 | 326 | 344 | **349** | 35.86 | 0.750 | 35.86 | 0.002 |
| 4 | 365 | 359 | 418 | 421 | **391** | | | | <0.001 (L) |
| 6 | 387 | 467 | 467 | 407 | **432** | | | | 0.399 (Q) |
| 14 | 537 | 520 | 407 | 602 | **517** | | | | 0.927 (Dev) |
| **Mean** | **419** | **421** | **404** | **444** | **422** | | | | |

**Conclusions** The use of a ZnO supplemented diet for 14 days immediately post-weaning did improve DLWG and FCR; however, no additive responses to growth-promotant levels of avilamycin (40 ppm) were observed. In addition, the use of all three supplemental diets enhanced villus height throughout the first 6 days post-weaning when a reduction in villus height is often characteristic. The results therefore suggest that feeding a ZnO supplemented diet maintains the structure of the small intestine post-weaning resulting in a greater absorptive surface area in the gut. Although supplemental ZnO does appear to stimulate feed intake, the enhanced growth observed is not solely a function of increased voluntary feed intake. This is in agreement with work conducted by Carlson *et al.,* (1999). Further reasearch is required to elucidate the mechanism for the feed intake response.

**Acknowledgements** The support of the MLC and SCA Nutrition Ltd is gratefully acknowledged.

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# Enhancement of the ability of the porcine colonic microflora to resist colonisation by *Salmonella poona*, in an *in vitro* intestinal simulation

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**Introduction** The ability of the porcine intestinal microflora to resist the establishment of pathogenic bacteria has been demonstrated previously (Hillman *et al*, 1994). Subsequent work has shown that certain intestinal lactobacilli react to the presence of a culture filtrate derived from a coliform pathogen by increasing their antagonistic activity towards that pathogen (Hillman and Robertson, unpublished), indicating the presence of a quorum-sensing or related mechanism. The current experiment was devised to determine whether a similar effect could be produced within the entire porcine colonic microflora, using an *in vitro* simulation system.

**Materials and Methods** Two simultaneous *in vitro* simulations of the porcine colon (Khaddour *et al*, 1998) were inoculated with fresh porcine faeces and allowed to stabilise for 4 days. For three subsequent days, one of the vessels was dosed daily with 20 ml of a culture filtrate of *Salmonella poona* (grown in nutrient broth and filtered under vacuum through 0.2 μm sterile cellulose nitrate membrane filters). Both vessels were then inoculated with a naturally occurring nalidixic acid resistant strain of *S. poona* to at least $10^6$ viable cells per ml. The decline of the strain in each vessel was followed by enumeration on XLD medium supplemented with 20 $gl^{-1}$ nalidixic acid to prevent the growth of other coliform species. Black colonies appearing on this medium within 24 h at 39°C were taken to be representatives of the inoculated strain. Samples taken before inoculation showed no growth, demonstrating that the test strain was absent from the original fermentor contents. Enumeration was performed immediately after inoculation, then at 6 h, 24h, 48 h and 72 h. The experiment was repeated to provide triplicate data, alternating the treatment and control simulators. Data were transformed to log10 to provide linear decline rates and were analysed both by comparing datapoints and by ANOVA on the rates of decline (determined by linear regression using MINITAB).

**Results**

Table 1. *Salmonella poona* (log10-transformed cfu per ml) over 72 h after infection, with or without pretreatment with filtrate.

| Time (h) | Test | Control | P |
|---|---|---|---|
| 0 | 6.8 | 6.7 | 0.816 |
| 6 | 6.8 | 6.6 | 0.717 |
| 24 | 5.9 | 6.3 | 0.435 |
| 48 | 5.2 | 5.9 | 0.354 |
| 72 | 4.7 | 5.5 | 0.196 |

Data represent means of triplicate log10-transformed counts of *S. poona* at each time point.

Table 2. Rates of decline of *S. poona* under test and control conditions in individual experiments.

| Run | Test | $r^2$ | Control | $r^2$ |
|---|---|---|---|---|
| A | 0.75 | 0.99 | 0.35 | 0.96 |
| B | 0.60 | 0.86 | 0.43 | 0.94 |
| C | 0.91 | 0.93 | 0.41 | 0.92 |
| Mean | 0.75 | | 0.40 | |
| SD | 0.154 | | 0.038 | P=0.017 |

Data represent the rate of decline of *S. poona* (log10 units per ml per day) within each of the three experiments.

Direct comparison of data at each time point (table 1) shows a divergence between the test and control data over the course of the experiment, although statistical significance was not achieved with this data. This may be due to variation in the values of the initial inoculum which has resulted in a wide variance in the data obtained. If, however, we examine the rates of decline of *S. poona* under both test and control conditions (table 2), these can be seen to show greater consistency, demonstrating a significant ($P<0.05$) increase in the removal of *S. poona* from the treated system.

**Conclusion** The results indicate that a compound produced by *S. poona* either directly acts to reduce the growth rate of the pathogen, or is detected by other components of the intestinal microflora, resulting in the production of an antagonistic effect to this pathogen within the microflora. The current experiment cannot determine which compound(s) or which intestinal species are involved in this reaction. Nonetheless, the experiment has demonstrated that it is possible to enhance the resistance of the intestinal microflora to *S. poona* by applying a cell-free filtrate derived from a culture of this pathogen.

**Acknowledgement** Funding for this work was provided by SEERAD under RO 601028.

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# The effect of dry matter concentration of liquid diets on the growth performance of grower-finisher pigs.

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**Introduction.** There is a considerable body of information on the voluntary feed intake of pigs fed on dry diets. However, there is a paucity of information on the factors affecting feed intake of pigs fed liquid diets. Pigs have a limit to their volumetric intake and will normally maximise dry matter intake when fed dry diets, *ad libitum*. For pigs fed dry feed the requirement for water per unit of dry matter will be dependent upon the composition of the feed and the requirement for renal clearance of nitrogen and minerals. What is not clear, is whether presenting the feed mixed with water results in excessive water intake that produces a point loading of the stomach, which in turn limits voluntary feed intake, i.e. whether physical bulk limitations override the normal homeostatic control. Therefore, it is not possible to extrapolate from data generated with dry fed pigs and anticipate voluntary intake of pigs fed dilute liquid diets. The aim of this study was to determine the effect of dry matter concentration of liquid feed on feed intake and growth performance of grower-finisher pigs.

**Methods**. A study was conducted according to a 3 x 4 factorial design. Factor 1 was the live-weight of pig (35, 55 or 75 kg) and factor 2 was the oven-dry matter (ODM) concentration of the diet (89, 178, 267 or 356 g $kg^{-1}$ feed). The study was conducted using 288 pigs (from PIC line 15 hybrid sows x PIC 'Meatlink' boars) in six blocks of 48 pigs. Each block comprised 16 pigs from each target weight category which were randomly allocated to one of four dietary dry matter treatments and housed in pens of four (2 male, 2 female per pen) according to treatment. Commercial diets appropriate for the weights of pig comprised (g $kg^{-1}$ 35, 55 and 65 kg pigs respectively), wheat (398, 392, 406); barley (150,150,175); 'Hipro' soya (106,167,114); full fat soya (116, 61, 0); wheatfeed (100,100,143); rapeseed (50, 85, 130); fishmeal (37.5, 0, 0); fat (20,20,10); limestone (11, 12.5, 11.4); dicalcium phosphate (3, 4.7, 3) lysine (3, 2,2) salt (1.7, 3, 2.6); methionine (0.004, 0, 0) and vitamin premix (2.5,2.5,2.5). The diets were mixed with water to give four dry matter concentrations, 89, 178, 267 or 356 g ODM $kg^{-1}$ feed. The diets were sanitised with 300 ppm chlorine dioxide for 1 h prior to feeding. The pigs were fed *ad libitum*. The troughs replenished with fresh feed daily. Unconsumed feed was removed from the trough, weighed back, and dry matter feed intake estimated by drying samples of unconsumed feed to constant weight at 105°C. Additional water was freely available in each pen from metered drinkers. The pigs remained on trial for 10 days, (three days acclimatisation followed by seven days of data collection). Live weight at the start and finish of the collection period, daily *ad lib.* dry matter feed intake and daily water use from drinkers per pen of 4 pigs were recorded. Statistical analyses were performed by polynomial analysis of variance using Genstat 5.

**Results.** The ODM concentration of the diet had a significant effect on ADG ($P<0.001$) and ADFI ($P<0.001$) but not on FCR ($P>0.05$) (Table 1). Average daily feed intake and average daily gain were significantly reduced in pigs fed dietary ODM concentrations of 89 g $kg^{-1}$ compared with 178, 267 or 356 g $kg^{-1}$. The use of water from drinkers and total water consumed was significantly affected by dietary ODM ($P < 0.001$). Even when fed diets containing only 89 g $kg^{-1}$ ODM pigs still took 0.15 litre water per day from drinkers. The pig weight group significantly affected ADG ($P<0.001$) and water intake ($P<0.001$) but not ADFI or FCR ($P>0.05$). There was a significant interaction between dietary ODM and pig weight for water use from drinkers ($P<0.01$) but not for any of the other parameters measured.

Table 1 Growth performance and water use of pigs from different weight categories fed diets of different dry matter concentration.

| | ODM of Diet (g $kg^{-1}$) | | | | | Pig weight group (kg) | | | | *P* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 89 | 178 | 267 | 356 | s.e.d. | 35 | 55 | 75 | s.e.d. | Diet (A) | Wt (B) | AxB |
| ADG (kg $d^{-1}$) | 0.46 | 0.73 | 0.84 | 0.83 | 0.093 | 0.69 | 0.65 | 0.78 | 0.08 | 0.002 | <0.001 | 0.402 |
| ADFI (kg $d^{-1}$) | 1.87 | 2.30 | 2.38 | 2.31 | 0.136 | 1.61 | 2.34 | 2.73 | 0.118 | <0.001 | 0.269 | 0.392 |
| FCR | 7.03 | 3.92 | 3.10 | 3.06 | 2.510 | 2.97 | 5.59 | 4.27 | 2.173 | 0.350 | 0.490 | 0.954 |
| Water drinkers (l $d^{-1}$) | 0.15 | 0.61 | 0.58 | 2.42 | 0.211 | 0.67 | 1.21 | 0.95 | 0.94 | <0.001 | 0.004 | 0.006 |
| Total water (l $d^{-1}$) | 18.21 | 11.35 | 7.21 | 6.66 | 1.226 | 8.32 | 12.21 | 12.10 | 0.658 | <0.001 | <0.001 | 0.597 |

ADFI and FCR calculated as oven dried matter feed intake and oven dried matter feed consumed per kg gain. ODM of air dried feed was 890 g $kg^{-1}$

**Conclusions** The results showed that there was little difference in growth rate when pigs were fed diets containing 267 or 356 g $kg^{-1}$ ODM concentration. Although not statistically significant, reducing the ODM from 356 to 178 g $kg^{-1}$ reduced feed intake and daily gain but more importantly almost doubled the water consumption and hence effluent output per kilogram of gain. Further reducing the ODM to 89g $kg^{-1}$ dramatically reduced feed intake significantly reduced growth rate and resulted in a six-fold increase in water use and hence effluent output per kilogram of gain.

**Acknowledgements** The financial support of DEFRA and the MLC is gratefully acknowledged.

# The effect of dry matter concentration on component digestibility and retention in growing/finishing pigs fed diets varying only in water:dry matter ratio

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**Introduction** There is potential for improving the growth performance of pigs through a better understanding of factors that influence the digestibility of nutrients and energy in liquid feeds. These factors include dry matter content and the size and distribution of particles of the dry feed components within the liquid diet. The objective of the current study was to determine if changing the dry matter concentration of liquid feeds affects the digestibility and retention of nutrients and energy in pigs growing from 35kg to 95kg live weight.

**Materials and methods** A metabolism study was conducted using ten male pigs, (modern commercial white hybrids); five were maintained on an *ad libitum* (AL) and five on a restricted (R, 0.85 *ad libitum*) feeding regime. For the duration of the trial animals were housed individually in pens and transferred into metabolism crates for each collection period. A single dry meal diet (**Table 1**) was used to make five experimental diets differing only in the ratio of water: dry matter (**Table 2**). Experimental diets were supplemented with titanium dioxide ($TiO_2$, solid phase marker, 1g/kg) and polyethylene glycol (PEG 4000, liquid phase marker, 20g/kg), and sanitised using Sanitech™ (12ml per litre, activated with 2g citric acid per 12ml Sanitech) to prevent fermentation. Each diet was fed to one AL and one R pig in each of 4 collection periods (conducted at mean pig live weights of 35, 55, 75 and 95kg). Water was available at all times from Arato 80 nipple drinkers, each fitted with a Kent PSM-L water meter to monitor individual water intake. Each collection period consisted of five days (minimum) acclimatisation followed by five days during which individual feed and water intake/waste was measured and a total collection of urine and faeces conducted, with collected material being stored at –20°C. Feed, faeces and urine samples were analysed for $TiO_2$, PEG 4000 and chemical components, and digestibility coefficients calculated for nutrients and energy.

**Table 1** *Diet formulation, g/kg (providing 14.00MJ DE/kg, 207g CP/kg, 10.25g AV Lys/kg)*

| Ingredient | Content | Ingredient | Content |
|---|---|---|---|
| Wheat | 392 | Fat Blend | 20.00 |
| Hipro soya | 167 | Limestone Flour | 12.50 |
| Barley | 150 | Dical Phosphate | 4.68 |
| Wheatfeed | 100 | Salt | 3.08 |
| Rapeseed "00" | 85 | Premix | 2.50 |
| Full fat soya | 61 | Lysine HCl | 2.00 |
| | | Vit E 50 | 0.05 |

**Table 2** *Experimental diets, g/kg*

| Diet | A | B | C | D | E |
|---|---|---|---|---|---|
| Form of feed fed | ——— | liquid | ——— | | dry |
| g air dry feed/kg feed fed | 200 | 250 | 300 | 400 | 1000 |
| ml water/kg feed fed | 800 | 750 | 700 | 600 | 0 |
| water:feed ratio of feed fed | 4:1 | 3:1 | 2.3:1 | 1.5:1 | 0 |
| water:DM[a] ratio of feed fed | 4.6:1 | 3.5:1 | 2.7:1 | 1.7:1 | 0.1:1 |

[a]oven dry matter content of air dry feed was 869g/kg

**Results** Data were analysed as a 2 (rate of feeding) * 5 (ratio of water to dry meal, with linear and non-linear contrasts assessed) factorial model. No significant differences were found between feeding regimes (*NS*) or collection periods (*NS*) for any of the factors evaluated, and so the results presented are the mean of both AL and R pigs over four collection periods. Mean feed intake (g/day/kg LWT) for diets A-E was 166, 149, 126, 93 and 36 respectively (*s.e.d.=11.4, P=<0.001*), whilst the corresponding mean oven dry matter intake was 29, 32, 33, 32 and 31 (*s.e.d.=3.6, NS)*. Water intake from the drinkers (ml/day/kg LWT) decreased significantly with increasing water concentration in the feed (mean = 18, *s.e.d.=9.8, P<0.001*) although total water intake (including that consumed in the feed) increased (mean = 96, *s.e.d.=13.5, P<0.001*); water retention coefficient decreased (*mean = 0.317, s.e.d.=0.0462, P=0.001*) with no significant differences in absolute retention of water, GE or N (*NS*). Neither faecal fresh matter (FM) output (mean = 22gFM/day/kg LWT, *s.e.d.=3.2, NS*) nor faecal moisture content (mean = 306gDM/kg FM, *s.e.d.=12.1, NS*) differed significantly between diets. Data presented in **Table 3** show that there were no significant differences in component digestibility between diets.

**Table 3** *Coefficients of component and energy digestibility in growing pigs fed diets differing only in DM concentration*

| Diet | A | B | C | D | E | Mean | s.e.d. | P | Significance |
|---|---|---|---|---|---|---|---|---|---|
| DM | 0.776 | 0.770 | 0.770 | 0.777 | 0.769 | 0.772 | 0.0157 | 0.986 | *NS* |
| GE | 0.755 | 0.747 | 0.747 | 0.754 | 0.750 | 0.751 | 0.0172 | 0.991 | *NS* |
| CP | 0.824 | 0.817 | 0.814 | 0.824 | 0.812 | 0.818 | 0.0184 | 0.970 | *NS* |
| Oil | 0.670 | 0.672 | 0.688 | 0.700 | 0.680 | 0.682 | 0.0269 | 0.798 | *NS* |
| NDF | 0.684 | 0.676 | 0.681 | 0.699 | 0.689 | 0.686 | 0.0258 | 0.913 | *NS* |
| ADF | 0.664 | 0.666 | 0.672 | 0.688 | 0.666 | 0.671 | 0.0472 | 0.985 | *NS* |

**Conclusions** Differences in the liquid feed intake of growing pigs are attributable only to the water fraction of the diet since there were no significant differences in dry matter intake between dietary treatments. Results show that variations in the dry matter concentration of liquid feeds have no consequence for energy/nutrient digestibility and retention in growing pigs. Additionally, increasing the water content of the liquid feed results in increased slurry volume although the concentration of waste components is decreased, suggesting that the overall environmental impact of liquid feeding is likely to be similar over a wide range of water to dry matter ratios. In summary, results of the current study show that the nutritional and environmental effects of varying the dry matter concentration of pig feed over the range of 174 to 869g DM/kg feed are negligible. **Acknowledgements** The financial support of DEFRA and the MLC is gratefully acknowledged.

# Influence of feeding fermented liquid feed on faecal bacterial flora and selected colostrum parameters of lactating sows

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**Introduction**. Farrowing and lactation are two of the most critical phases of pork production. A relatively high proportion of pig losses occurs during these periods. Rapid bacterial colonisation of piglets' sterile gut and underdeveloped immune system represents a very dangerous combination of events. The most significant factors affecting the microflora of the piglet's gut is its mother and the environment into which it is born. Therefore, management interventions and nutritional regimes that influence the microbiology of the sow's faeces in a beneficial way are likely also to influence the neonate. Work at the University of Plymouth and at Foulum in Denmark has shown that feed may be fermented successfully with lactic acid bacteria (LAB) and that this process reduces the number of salmonellae and coliforms in the feed and consequently in the lower gastrointestinal tract (Brooks *et al*. 2001). Recent studies strongly support the hypothesis that orally administered LAB stimulate the immune system, both at the local and systemic level. This combination of effective immunity and reduced level of environmental contamination with faecal pathogens can lead to improved management of sows for increased litter size and weight at weaning time.

**Materials and methods**. A study was conducted according to a randomised block design, with six replicates. A replicate consisted of 3 animals each fed one of three dietary treatments: a) fermented liquid feed (FLF); b) non-fermented liquid feed (NFLF); c) dry feed (DF). A rifampicin resistant *Lactobacillus plantarum* (PC-81-11-02, Alltech Inc., Kentucky), spontaneous mutant, was used as a starter culture. After 24-hours sanitation with chlorine dioxide (Sanitech 2%; Alltech Inc., Kentucky) the feed was inoculated with liquid lactobacillus starter culture to give a final concentration of between 6 and 7 $\log_{10}$ cfu $ml^{-1}$ liquid feed. The inoculated feed was fermented for 96 hours at 30$^{o}$C. A total of 18 multiparous sows (Large White x Landrace) were fed twice a day for a period of 2 weeks before their anticipated farrowing date and for 3 weeks after farrowing. Fresh faecal samples were collected from the rectum of each sow 7 days before parturition and 7 days after. Faecal samples were also collected from the piglets after 7 days of suckling. LAB and coliforms were analysed in each faecal sample by standard methods. SCFA analyses of faecal samples were conducted by HPLC. Colostrum samples were collected on the day of parturition by manual milking. Rat intestinal epithelial cells (IEC-6) and pig lymphocytes were used to investigate mitogenic activity of colostrum samples. The bacteria count per gram of faeces was further log transformed, tabulated, and statistically analysed by ANOVA. Mitogenic experiments were carried out in triplicate determinations and repeated at least twice. Significant differences between treatment means were compared by Tukey's HSD test. Statistical analyses were undertaken using Minitab v 10.2 (Minitab Inc., Pensylvania, USA, 1994).

**Results.**

**Table 1** Numbers of LAB and coliforms ($\log_{10}$ cfu $g^{-1}$) ± SEM in the sow's faeces 1 week before and 1 week after farrowing and in the piglet faeces.

| | *Sow before farrowing* | | *Sow after farrowing* | | *Piglets (1week old)* | |
|---|---|---|---|---|---|---|
| | LAB | Coliforms | LAB | Coliforms | LAB | Coliforms |
| FLF | 6.8 ±0.49[a] | 5.3±0.32[b] | 6.8±0.27 [a] | 5.0±0.40 [d] | 7.7±0.07 [a] | 7.5±0.13 [d] |
| NFLF | 7.5 ±0.24[a] | 4.8±0.26[b] | 6.4±0.38 [a] | 6.6±0.40 [c] | 7.6±0.10 [a] | 7.8±0.06 [c,d] |
| DF | 7.7±0.15 [a] | 5.3±0.33[b] | 6.5±0.26 [a] | 7.2±0.23 [b] | 7.3±0.13 [b] | 8.1± 0.10[c] |

[a,b,c,d] Within columns, means with a common superscript are not statistically different

**Table 2** Mitogenic activity of sow colostrum on IEC-6 cells and blood lymphocytes

| | IEC-6 | Lymph |
|---|---|---|
| FLF | 79329±3069 [a] | 1903± 204 [a] |
| NFLF | 75669± 3091 [a] | 1000± 97.6 [b] |
| DF | 53433± 1568 [b] | 1231± 61.4 [b] |

Data are expressed as a mean counts per minute (CPM)±SEM; [a,b]Within columns, means with a common superscript are not statistically different.

**Conclusions** This study showed that sow's gastrointestinal microflora as ell as the quality of colostrum produced could be influenced by the diet she receives. While the LAB population was not significantly affected by dietary treatment, significant differences in coliform population were observed in the sow's faecal samples taken 7 days after parturition (Table 1). Faeces excreted from FLF fed sows had significantly ($P<0.001$) lower numbers of coliforms compared with sows fed NFLF or DF. Subsequently, piglets from FLF-fed mothers excreted faeces that were significantly higher ($P<0.01$) in LAB and significantly lower ($P<0.001$) in coliforms than faeces from the piglets of DF-fed dams (Table 1). Analyses of colostrum samples showed that colostrum from FLF-fed sows had a significantly greater ($P<0.001$) mitogenic activity on both IEC-6 cells and blood lymphocytes compared with colostrum from DF-fed sows (Table 2). This means that colostrum from FLF-fed mothers may help speed up the maturation of the newborn's GI tract and immune system. Cell proliferation and differentiation are key elements in the efficient function of the GI tract and immune system. This improved colostral mitogenic activity could be explained by a higher concentration of nutrients and growth factors such as IGF-I, IGF-II and EGF, but the exact mechanisms by which that occurs remain to be determined. The study suggested that the use of fermented liquid feed could be an important tool in combating enteropathogens in the GITof healthy animals and could result in improved litter performance and profitability.

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# Effect Of L-Thyroxin Hormone (T4) On Compensatory Growth In Broiler Chicks

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**Introduction** Purpose of researches in feed restriction area is improvement of feed efficiency, decrease of carcass fat content and abdominal fat pad size (Plavnik and Hurvitz,1991).Birds after early life feed restriction have less maintenance requirements due to decrease of heat increment and decrease of basal metabolic rate and specific dynamic action of food (Forsum et al.,1981).In many investigations, compensatory growth have not observed (Summers et al.,1990).It seems administration of Thyroid hormone after feed restriction can induce compensatory growth. The objective of the present study was to investigate the effect of early feed restriction and L-Thyroxin administration after early feed restriction on compensatory growth in broiler chickens.

**Material and methods** Six hundred male and/or female day old chicks of a commercial strain (Hybro) were wing banded, weighted and randomly allocated to five treatment groups of each sex. There were 5 replicates of 12 male or female chicks in each treatment. Five treatments involved, one control group (No Restriction and No Hormone) and four restricted groups which were fed a mixture of 50:50 rice hulls and commercial starter diet from 4 to 11 days of age. Percent of trace minerals and vitamin premix in control and restriction diets were remained similar. All groups were fed the same diets from 11 to 56 days of age with the exception of the 4 restricted groups which their diets were supplemented with 0,1,2 and 3 ppm T4 from 11 to 28 days of age. Live body weight, feed intake, feed efficiency, body composition (protein, fat and ash), fat pad size at 49 and 56 days of age were determined. Analysis of variance and Duncan's new multiple range test were conducted using the General Linear Model procedure of SAS (SAS Institute, 1985) appropriate for a completely randomized design.

**Results** Live body weight of Non T4 Receiving Restricted birds was compensated on day 42 of age. But, the T4 treated birds after feed restriction period, showed a decrease in live body weight as the level of T4 increased. Daily feed intake up to 42,49 and 56 days was similar in control and only restriction (0ppm) groups and was significantly ($p < .05$) decreased by dietary T4 as the level of T4 increased. Feed efficiency in the only restricted birds and control group was similar and all T4 treated birds exhibited a poorer feed efficiency for 42 and 49 days of experimental period, but it was not significantly different for 56 days period. Carcass fat content of only restricted birds was significantly ($p< .05$) lower than of control birds but all T4 treated birds had a similar carcass fat with control group. Abdominal fat pad in only restricted birds at 49 days was significantly ($p < .05$) lower than of control birds. Although, all birds had a similar abdominal fat percentage at 56 days of age.

**Table 1** Effect of L-Thyroxin Hormone after feed restriction on growth performance of broiler chicks in 49 days of age

| Treat | Body weight(g) | Feed intake[1](g/d) | Feed efficiency [1] | Carcass fat as % wet carcass | abdominal fat as % LBW |
|---|---|---|---|---|---|
| Control | 2169.88[a] | 96.27[a] | 2.21 [b] | 30.08 [a] | 3.23 [a] |
| 0ppm | 2207.82 [a] | 95.52 [a] | 2.22 [b] | 22.52 [b] | 2.57 [b] |
| 1ppm | 2061.36[b] | 94.56 [a] | 2.37 [a] | 27.6 1 [a] | 2.73[ab] |
| 2ppm | 1998.29 [bc] | 91.01 [b] | 2.36 [a] | 29.44 [a] | 2.69 [ab] |
| 3ppm | 1935.73 [c] | 88.93 [b] | 2.38[a] | 29.29 [a] | 2.67 [ab] |
| SE | 22.412 | 1.011 | .026 | .689 | .331 |

. [abc] different superscript denote significant differences($p<.05$)

1- Without consideration of rice hulls

**Conclusions** With a period of moderate feed restriction in early of life, live body weight of birds was compensated in 42 days of age and in 56 days of age was numerically higher than control group (2.5%).T4 treated birds had a poorer body weight and other growth performances in overall period of experiment. Early life feed restriction caused significantly decrease in fat content of carcass and abdominal fat pad size.

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# A detoxification method of Khorasan cottonseed meal gossypol for broiler chicken rations

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**Introduction** Cotton is one of the main agricultural crops of Iran. The potential of cottonseed meal (CSM) for poultry nutrition is limited by the presence of gossypol, a toxic polyphenolic pigment. Gossypol exists in CSM in two forms, free and bound. Free gossypol, as defined by AOCS official methods, are those gossypol and gossypol derivatives that are soluble in aqueous acetone and are physiologically active. Bound gossypol is insoluble in ether, chloroform or aqueous acetone and for the most part is physiologically inactive (Berardi & Goldblatt, 1980). The objectives of the experiments reported herein were to study of gossypol reduction by dehydrated ferro sulfate( DFS) and lecithin and also the feeding value of a commercially processed CSM in broiler diets.

**Materials and methods** The following steps were used to evaluate the amount of free gossypol in CSM samples of Khorasan province. Samples of ( 1-2 Kg of ) CSM were taken from four oil extraction factories. Mashad cottonseed meal ( MCSM) had more crude protein(29.3%) and less crude fiber(22.5%), therefore this meal was used to study the broiler chicken performance. Total and free gossypol content of CSM samples were determined by HPLC (Hron et al. 1990). For determination the effect of dehydrated ferro sulfate and lecithin in lowering free gossypol the following treatments were used in the lab: 1- Control MCSM (with zero DFS), 2- MCSM with added DFS ( as equal amount of free gossypol), 3- MCSM with added DFS (as equal amount of total gossypol), 4- MCSM with added lecithin ( as 0.5 percent of MCSM on weight basis). In the *in vitro* experiment the broilers performance were studied by a factorially arranged (3 X 4) completely randomized design experiment consisting of four levels of MCSM (0, 5, 10, 20 % of diet) and three levels of DFS ( zero, equal to free gossypol and equal to total gossypol). Each treatment was consisted of 6 replicates of 8 day-old male Ross broiler chickens. Experimental diets (starter, grower and finisher) were isocaloric and isonitrogenous, according to NRC(1994).

**Results** Free and total gossypol in MCSM sample were determined to be 0.023 and 0.077 percent respectively. Addition of DFS equal to free gossypol , in the lab, lowered free gossypol to 0.0001 percent and addition of DFS equal to total gossypol and 0.5 percent lecithin lowered free gossypol to zero. Different levels of DFS and MCSM did not have any significant effect on daily feed intake, live weight gain, feed conversion and mortality of the broiler chickens up to 56 days of age.

**Conclusion** The result of the *in vitro* experiment demonstrate that addition of DFS and lecithin to MCSM can lower it's free gossypol to zero and also according to the *in vivo* experiment use of MCSM with 0.023 percent free gossypol, to 20 percent of the ration, don't have any undesirable effect on the broiler chickens performance.

**Acknowledgment** The authors wishes to acknowledge the funding for this work from the University of Mashad, Iran.

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# Effect of date of mating and housing on lamb growth, adipose tissue deposition and plasma leptin concentrations

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**Introduction** In lambs, the rapid increase in heat production after birth is due to initiation of nonshivering thermogenesis in brown adipose tissue (BAT). This occurs in conjunction with an increase in amount and activity of BAT specific uncoupling protein 1 (UCP1) (Clarke *et al*. 1997). UCP1 abundance and activity is low in fetal life but, within twelve hours of birth, there is an increase in the thermogenic activity of BAT and mRNA for UCP1. This ontogeny of UCP1 mRNA in BAT is very similar that of leptin, which is first detectable in the sheep fetus at 90 days gestation in fetal adipose tissue, its expression then increases up to term at 147 days (Yuen et al 1999). Leptin is a hormone which is thought to play a physiological role is in energy balance, it is primarily produced by white adipose tissue although there is evidence for its production in both brown adipose tissue and the placenta. Lambs born in the autumn are known to be smaller than those born in the spring (McCoard *et al*. 1997). It is not known if moderate changes in date of mating can influence birth weight or adipose tissue development. The present study aimed to determine whether date of mating could influence lamb birth weight, the abundance of BAT, UCP1, plasma leptin.

**Materials and Method** Thirteen triplet bearing ewes were divided into two groups according to their date of mating (Group 2 conceived two weeks, i.e. one cycle after Group 1 (ewes were mated from early November)). All ewes were group housed from 56 (Group 2) and 77 (Group 1) days gestation and offered straw ad libitum and a fixed amount of concentrate. After lambing, the ewes and their lambs were individually housed. One randomly selected lamb from each set of triplets was entered into the study and jugular venous catheters inserted to allow daily blood sampling. For the following seven days, the lambs were weighed blood sampled. On day seven, perirenal adipose tissue (PAT) was sampled from all lambs following euthanasia. Abundance of UCP-1 was determined by immunoblotting and values are expressed as a percentage of a reference sample included on all gels. Plasma leptin levels were determined using a radioimmunoassay. Results were analysed by Mann Whitney U-test and are presented as means and standard errors (SEM).

**Results**

**Table 1. Summary of the effect of time of mating on body weight, adipose tissue weight and plasma leptin**

| Group | Lamb birth weight (kg) | | Plasma Leptin (ng/ml) | | Weight of PAT On Day 7 (g) | | PAT g/kg Body Weight (g) | | Abundance of UCP1 (% of reference) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| Group 1 | 3.96 | 0.18 [a] | 2.22 | 0.24 | 22.2 | 2.44 [a] | 4.79 | 0.84 | 81.9 | 8.69 [a] |
| Group 2 | 5.18 | 0.19 | 1.72 | 0.31 | 37.5 | 5.43 | 4.91 | 0.63 | 58.0 | 6.91 |

a: Significant difference between the two groups at $p<0.05$ level.

The lambs born two weeks earlier in the season were smaller and grew at a significantly slower rate than the lambs born two weeks later (Group 1, 122 g/day; Group 2**,** 174 g/day ($P<0.05$)). Group one lambs also had less total adipose tissue but a greater abundance of UCP1 on day seven but similar plasma leptin compared to group two.

**Conclusion.** A two week delay in the date of mating resulted in an increase in both lamb birth weight and adipose tissue deposition whilst there was a decrease in the abundance of UCP 1 and no change in the plasma leptin levels. The cause and possible long term consequences of these differences in UCP1 abundance, lamb weight, colonic temperature and plasma leptin levels for lambs born 2 weeks apart remain to be determined.

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**Acknowledgements**

S. Pearce was supported by a BBSRC Postgraduate Studentship

# Leptin interrelationships to energy-related metabolites and hormones in fed and fasted sheep

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**Introduction** A review of published leptin data for growing lambs, older ewes and mature dairy cows in late lactation showed that only 0.30-0.37 of the variation in blood leptin concentration was explained by differences in body fat variably expressed as % of liveweight (LW), backfat thickness and body condition score (BCS) respectively (Wylie *et al*., 2002). In dairy cows between 15d and 226d postpartum, Wylie *et al* (2002) observed no overall correlation between leptin at slaughter and lipid expressed as % of LW, empty body weight or carcase weight and only a weak correlation in cows in mid-lactation. Losses of fat during early lactation may 'uncouple' the link between leptin and fat and produce a bias across all of lactation. Another explanation is that leptin may be more closely linked with lipogenesis than with the amount of stored fat. This study revisits some metabolite and hormone data from a previous investigation of IGF-1 changes in fed, fasted and re-fed sheep in the light of more recently obtained leptin concentrations in the same animals.

**Methods and materials** In a study conducted in 1991 (Wylie, 1995), five wether lambs (mean initial LW = 39 kg; initial BCS < 2) were fed at a level (M) sufficient only to maintain initial LW for 21 days prior to a 5d fast (water only) followed by re-alimentation to 2M intake via 0.33M (4d), 0.66M (4d), M (6d), 1.5M (5d) and 2M (5d). The sheep were held at 15°C in an open-circuit respiration chamber between d15 and d50 and then for a further 14d at 2M. Blood was sampled weekly outside the chamber, daily during the 5d fast and on the final day of all other dietary periods. Sera were analysed for IGF-1, insulin, β-hydroxybutyrate (BOHB), NEFA and urea within 3 months. Sub-samples kept at –20°C were retrospectively analysed for leptin ten years later using a newly developed in-house bovine-ovine specific leptin radioimmunoassay (Wylie *et al*., 2000). Hormone and metabolite concentrations were correlated using Genstat 5.

**Results** Profiles of changes in mean serum IGF-1 and leptin concentrations are shown in Fig 1 while Table 1 shows the strength of the relationships between some energy-related metabolites and hormones. The sharp fall in IGF-1 on fasting and the strong correlation of insulin and IGF-1 (second only to insulin and glucose) reflects the role of specific nutrients (via insulin) in sustaining blood IGF-1 levels. The absence of a significant fall in leptin during fasting, and the earlier increase in IGF-1 than in leptin upon re-alimentation, is consistent with an association of IGF-1 with protein synthesis (an essential process) and of leptin with the more opportunistic process of lipid synthesis.

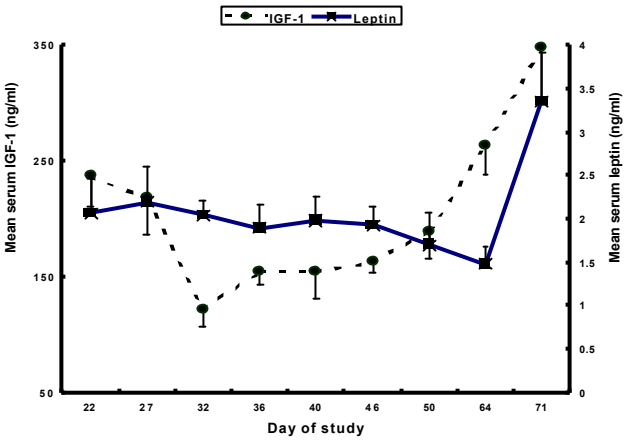


**Figure 1**. Changes in sheep serum leptin and IGF-1

**Table 1**. Correlations (r) of serum leptin with other energy related hormones and metabolites in fed and fasted wethers

| | Leptin | Insulin | IGF-1 | glucose | BOHB | NEFA |
|---|---|---|---|---|---|---|
| Leptin | 1.00 | | | | | |
| Insulin | 0.773 | 1.00 | | | | |
| IGF-1 | 0.604 | 0.870 | 1.00 | | | |
| Glucose | 0.845 | 0.972 | 0.830 | 1.00 | | |
| BOHB | -0.369 | -0.205 | 0.238 | -0.314 | 1.00 | |
| NEFA | -0.139 | -0.651 | -0.674 | -0.534 | -0.122 | 1.00 |

**Discussion**

The data confirms the clear associations between selected nutrients, insulin, IGF-1 and leptin in ruminants. Differences in the temporal profiles of leptin and IGF-1 concentrations during re-alimentation of fasted animals are consistent also with an association of leptin with lipogenesis but do not provide experimental proof of this hypothesis.

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# Exploration of somatotropic axis, leptin, insulin and blood biochemical parameters in ewes naturally affected with scrapie

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**Introduction** Scrapie is an ovine sub-acute transmissible spongiform encephalopathy (TSE) caused by unconventional transmissible agents. In several species, TSE are associated to major endocrinopathy, such as hyperinsulinemia and hypercorticism (Carp *et al*, 1990, Gayrard *et al*, 2000). Cachexia is commonly observed in the clinical phase of the prion disease. Our objective was to investigate if scrapie is associated to alterations of GH axis, leptin, insulin and metabolic parameters. In addition, central adrenergic system being affected in TSE (Braun *et al*, 1999), we investigated a possible alteration of α2-adrenergic control of GH axis associated to the prion disease.

**Materials and methods** Blood samples were collected every 30 min to determine the 24-h spontaneous pattern of GH plasma concentrations. GH response to intravenous administration of xylazine, an alpha2-agonist (0.15mg/kg) was determined in seven control and seven scrapie-affected ewes. For other determinations blood, samples were obtained before and 3 hours after morning meal. The ewes were tied in metabolism cages and received hay *ad lib* and two meals of 260 g of concentrates daily. Feed intake was not modified by the disease. The scrapie diagnosis was performed by histopathology. Body weights (mean ± SD) were 37±4 kg and 40±5 kg for scrapie-affected and control ewes, respectively. Plasma biochemical parameters and leptin were assayed by enzymatic methods or radioimmunoassay, respectively (Ferlay & Chilliard, 1999, Delavaud *et al*, 2000). In a separate period, in addition to plasma leptin concentrations, we assayed leptin in CSF sampled through a cannula inserted into a lateral ventricle. The influence of the disease and of meal on hormone and metabolite concentrations was assessed using ANOVA.

**Results** Plasma GH concentrations of scrapie-affected ewes tended to be greater than those observed in healthy ewes (mean±SD, 27.6±14.7 versus 16.8±12.6ng/ml), but the difference was not significant (P>0.1). Xylazine induced a transient and low increase in GH concentrations, with large interindividual variations. Mean plasma concentrations of some metabolites are given in Table 1. Plasma glucose and urea concentrations of scrapie-affected ewes were greater than those observed in healthy ewes (P≤0.05 and P=0.07 respectively). Mean insulin concentrations from diseased ewes were 2 fold higher compared with that of healthy ewes (P=0.07), whereas plasma IGF-1, leptin and 3-OH-butyrate concentrations were not affected by the prion disease. Post-prandial plasma NEFA concentration was lower in diseased ewes (P<0.05). CSF leptin concentrations were related to plasma leptin (r=+0.87; P<0.05) and unaffected by the prion disease (mean±SD, 1.6±0.9 and 1.3±0.4 ng/ml in 3 scrapie affected ewes and in 5 control ewes, respectively).

**Table 1** Plasma concentrations (mean±SD) of IGF-1, leptin, insulin, glucose, NEFA, 3-OH-butyrate and urea in 7 scrapie-affected ewes and in 7 control ewes, sampled before and 3 hours after morning meal

| | scrapie | | control | | Effect of scrapie |
|---|---|---|---|---|---|
| | before | after | before | after | P≤ |
| IGF-1 (ng/ml) | 133±61 | ND | 101±51 | ND | NS |
| leptin (ng/ml) | 3.7±0.31 | 3.6±0.27 | 4.3±1.1 | 4.2±0,9 | NS |
| Insulin (µUI/ml) | 24±13 | 28±19 | 11±3 | 15±7 | 0.07 |
| Glucose (g/L) | 0.74±0.1 | 0.74±0.08 | 0.58±0.06 | 0.57±0.07 | 0.005 |
| NEFA (mmol/l) | 0.36±0.19 | 0.13±0.07 | 0.29±0.19 | 0.30±0.28 | NS [1] |
| 3-OH-butyrate (mmol/l) | 0.34±0.11 | 0.41±0.09 | 0.42±0.24 | 0.43±0.17 | NS |
| Urea (g/l) | 0.32±0.06 | 0.33±0.06 | 0.23±0.11 | 0.24±0.11 | 0.07 |

[1] Interaction meal*disease (P< 0.005) ND : not determined

**Conclusions** Collectively, these results suggest that scrapie does not change plasma nor CSF leptin concentrations but induces different hormonal and metabolic disorders, in particular, hyperinsulinemia, hyperglycemia, hyperuremia and a trend to higher GH secretion. The increase of urea concentrations could be related to myolysis or to renal disturbance. Hyperglycemia and hyperinsulinemia could be a consequence of the major hypercorticism shown in scrapie affected ewes (Gayrard *et al*, 2000) and could reflect a syndrom of resistance to insulin.

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# Hourly measurement of plasma leptin and cortisol concentrations in non-pregnant ewes under group housing conditions over 23 hours

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**Introduction** The closely related actions of cortisol and leptin (Ahima & Flier, 2000) are involved with appetite, tissue growth and maturation, energy balance and weight deposition so that resistance to either may lead to obesity. In normal weight humans, plasma leptin and cortisol exhibit diurnal variation, peaking during darkness and late afternoon (respectively). In sheep, the literature consensus is that plasma cortisol levels are greatest during daylight. Ovine plasma leptin is also reported to vary in response to photoperiod-driven changes (Bocquier *et al*., 1998) and such as alterations in voluntary feed intake as daylength changes. Daily circadian patterns, however, are thought to be entrained by the time of daily feed presentation (Marie *et al*., 2001). The aim of the present study was to investigate the diurnal variation in ovine plasma leptin in unrestrained animals with ad-lib access to hay and water in relation to their plasma cortisol profile over the same period. Remote blood sampling was employed in order to reduce sampling stress that would affect the animals' plasma physiology.

**Materials and Methods** Six mature Border Leicester ewes were group-housed in a naturally-lit barn during March (12h daylight) with *ad-lib* access to hay and fresh water and a daily concetrate pellet ration was fed at 09:00. The animals were harnessed to automatic blood sampling equipment (ABSE) (Goddard, Gaskin & Macdonald, 1998) that took hourly jugular vein blood samples with operator interference only needed after 14h to replace full tubes. Plasma cortisol was measured using a commercial kit (Coat-a-Count, Euro DPC Ltd., Gwynned, UK) and leptin was assayed with an ovine-specific double antibody radioimmunoassay, as developed by Delavaud *et al* (Delavaud *et al*., 2000).

**Results** Repeated measure analysis on both leptin and cortisol data from 6am until 2pm did not reveal a difference in either concentration due to feeding at 9am ($p>0.3$). A one-way ANOVA did not reveal an effect of either photoperiod or plasma cortisol concentrations on plasma leptin levels ($p>0.6$). Similar analysis on plasma cortisol data, however, found that concentrations were significantly greater during daylight hours ($p<0.05$). Finally, cross-correlation analysis of cortisol and leptin data within each ewe failed to reveal correlation between the two over time. These data are shown in Fig.1.

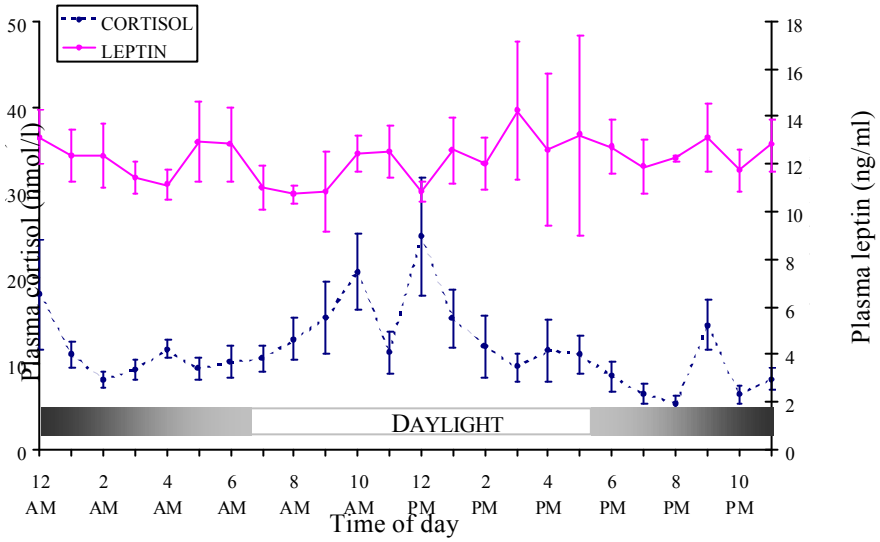


**Figure 1** Average (±SEM) plasma concentrations of leptin (ng/ml) and cortisol (nmol/l) in six ewes measured in 2 x 14-hour periods by automatic blood sampling equipment. Hay and water were provided ad-libitum. Feed concentrate pellets were provided at 09:00h.

**Conclusions** These data suggest that within a day, hourly fluctuations in plasma cortisol levels do not alter plasma leptin concentrations in sheep. Plasma cortisol levels alone were greater during daylight than at night and both leptin and cortisol levels were apparently unaffected acutely by time of pellet feed intake.

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JD was sponsored by a M.A.F.F. PhD studentship; SP was sponsored by a BBSRC PhD studentship. The project was sponsored by grants from The Society for Endocrinology and The University of Nottingham.

# Absence of plasma leptin or metabolite variation after subcutaneous melatonin release in adult ewe

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**Introduction** Leptin is mainly secreted by adipose tissue and plays an important role in body homeostasis and in a great number of physiological functions, particularly in reproduction. Sheep is a seasonal ruminant whose reproductive period is initiated during decreasing day lengths. Leptin expression and secretion have shown to be decreased during short days, independently of adipose cell size, feed intake and ovarian activity (Bocquier *et al*., 1998). The mechanism by which leptin could be modulated is not well understood (Chilliard and Bocquier, 2000). As nocturnal pineal melatonin secretion is increased during short days, it appears as a potential mediator in the regulation of plasma leptin by photoperiod. The aim of this study was to test this hypothesis by comparing plasma leptin and metabolite concentrations in adult ewes exposed to long days treated (M), or not (LD), with melatonin after 20 and 69 days.

**Materials and methods** Fourteen adult ewes (4.5 years old on average) were fed daily with 0.45 kg of concentrate and ad libitum straw. They were initially exposed to 70 short days (8 h light/day) and then to long days (16h light/day) until the end of the study. Six of them received 4 subcutaneous Regulin® implants containing 18 mg of melatonin each (CEVA Santé Animale, Libourne, France). Following the implantation, daytime plasma melatonin levels were equal to 202±24 pg/ml compared to 14±4 pg/ml in untreated animals. For all ewes, plasma samples were collected 15 days before, and 20 and 69 days after melatonin treatment. Leptin and prolactin concentrations were assayed by ovine-specific RIAs (Delavaud *et al*., 2000; Kann, 1971). Glucose, non-esterified fatty acids (NEFA), ß-OH-butyrate and urea concentrations were determined enzymatically using an autoanalyser (ELAN, Merck-Clévenot, France). Mean plasma measurements were adjusted with covariates obtained 15 days before treatment, when significant.

**Results** No difference in plasma leptin, glucose, NEFA, ß-OH-butyrate and urea concentrations was observed after 20 or 69 days of a melatonin treatment that inhibited prolactin secretion by about 50% (Table 1 or not shown).

**Table 1** Plasma leptin, prolactin and metabolite concentrations in ewes exposed to long days and treated (M), or not (LD), with melatonin during 20 and 69 days

| | LD-20 days | | M-20 days | | LD-69 days | | M-69 days | |
|---|---|---|---|---|---|---|---|---|
| | Mean | s.e.m. | Mean | s.e.m. | Mean | s.e.m. | Mean | s.e.m. |
| Leptin (ng/ml) | 9.5 | 0.5 | 9.3 | 0.6 | 7.0 | 0.3 | 7.1 | 0.4 |
| Prolactin (ng/ml) | 128 | 16 | 53** | 8 | 115 | 14 | 78* | 9 |
| Glucose (mmol/l) | 3.2 | 0.03 | 3.4 | 0.03 | 2.7 | 0.02 | 2.8 | 0.02 |
| NEFA (µmol/l) | 70 | 4 | 88 | 5 | 109 | 6 | 122 | 8 |
| ß-OH-butyrate (mmol/l) | 0.42 | 0.01 | 0.44 | 0.01 | 0.50 | 0.01 | 0.45 | 0.02 |

*,**, $P < 0.08$ or 0.01 for M *vs* LD when compared by Student's t-test, respectively

**Conclusions** The absence of melatonin effect on ewe plasma leptin seems to contradict results obtained in rats (Rasmussen *et al*., 2001) for physiological increases in plasma melatonin in both species. In the rat study, the decrease in plasma leptin after melatonin treatment was associated with a decrease in body fat content and insulinemia, suggesting a modulation of adipose tissue metabolism. Short days decreased sheep plasma leptin, whereas NEFA levels were increased and some adipose tissue lipogenic activities increased during long days (Bocquier *et al*., 1998, Faulconnier *et al*., 2001). However, in the present study, plasma NEFA were not affected by melatonin. This suggests that melatonin and prolactin are not the key factors implicated in the regulation of sheep plasma leptin and NEFA by photoperiod.

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# Post-partum blood leptin concentrations and energy balance in first calving dairy heifers

A.R.G. Wylie[1,2], D.J. Devlin[1], T.W.J. Keady[3], C.S. Mayne[1,2,3], R.E. Agnew[1,2,3] and T.Yan[3]

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**Introduction** Blood concentrations of leptin, the first of several recently-discovered adipocyte proteins, putatively signal the extent of fat energy reserves to the hypothalamus in mammals and help regulate food intake and reproductive activity (through control of GnRH release). In early post-partum dairy cows, the rapidly increasing milk energy output and slowly increasing feed energy intake produce a negative energy balance (-ve EB) that is met by mobilisation of adipose fat stores and the severity and/or duration of the –ve EB may delay resumption of normal ovarian activity. The objective of the current study was to compare post-partum EB and serum leptin concentration profiles in Holstein-Friesian and Norwegian Red heifers, both of high genetic merit within their breed.

**Materials and methods** Sixty-four Holstein-Friesian (HF) and Norwegian Red (NR) first calving heifers ($n$=32 of each) were blocked into pairs within breed immediately after calving and allocated equally and at random to either a low or high diet of grass silage and concentrates at an inclusion of 0.60 (H) or 0.30 (L) and 0.50 (H) or 0.20 (L) for days 1-100 and 101-200 respectively of lactation. Milk output and feed intake (via Calan gates and computerised load-cells) were used to provide estimates of EB (MJ/d) for each animal (Yan *et al*. (2002). Heifers were blood sampled fortnightly from 2 wks postpartum and leptin was determined using an improved bovine/ovine specific RIA (Wylie *et al*., 2000).

**Results** There was a clear difference in mean EB (across breeds) between the H and L diets offered in the current study (Fig.1) but no difference in mean EB between breeds until 22 weeks post-partum (Fig.2). Leptin profiles for the two breeds (Fig 3) revealed a lag in the onset of the postpartum rise in leptin in HF heifers and differences in mean leptin concentrations between the breeds were significant at weeks 6, 8 and 10 post-partum. Leptin concentration profiles over weeks 4 to 24 in the NR and weeks 10 to 30 in the HF heifers were strongly correlated ($R^2$= 0.87).

Fig 1. Mean EB (MJ/d) in heifers on high (H) and low (L) diets

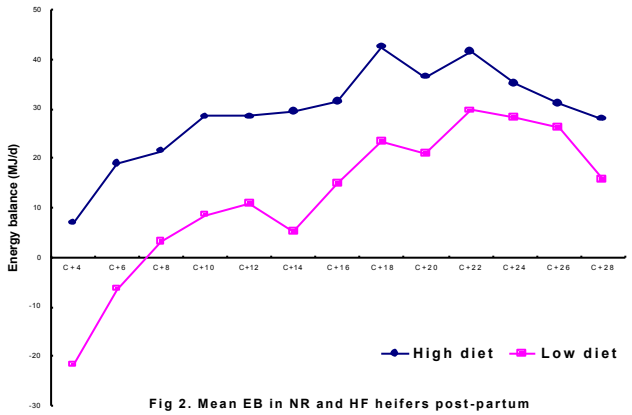



Fig 2. Mean EB in NR and HF heifers post-partum

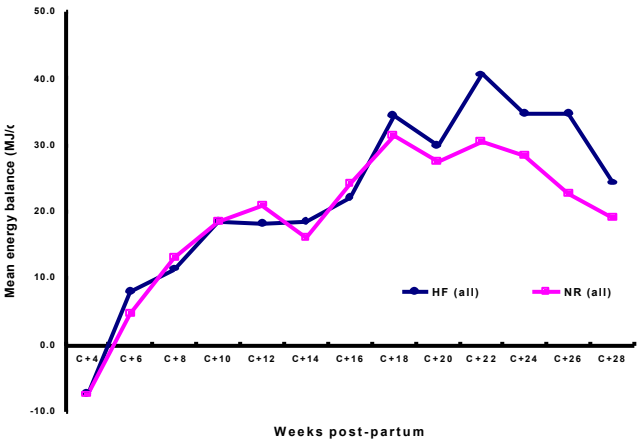
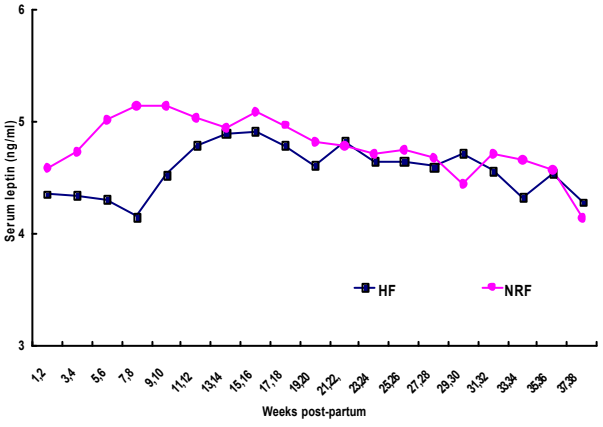



Fig 3. Mean post-partum serum leptin concentrations in HF and NRF heifers




**Conclusion** The serum leptin profiles were consistent with a preferential post-partum drive for milk production in the Holstein-Friesians and for tissue growth in the Norwegian Reds. The lag in the onset of the postpartum serum leptin increase in the Holstein-Friesian heifers may have physiological relevance for reproductive activity in this breed.

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# Plasma leptin in transition dairy cows. Effects of body fatness, ambient temperature and dietary factors

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**Introduction** In ruminants plasma leptin is increased with increasing body fatness. Leptin acts on hypothalamus to decrease food intake and increase energy expenditure. It is possible that leptin has a key role in transition from pregnancy to lactation of dairy cows. The objective of the present work was to investigate the pattern of plasma leptin concentration, as well as its relationship with other hormones and metabolites and dairy cow performance.

**Materials and methods** Experiment 1: 24 Holstein-Friesian cows were used in a continuous 2x2 factorial design. Experimental factors were feeding (F) method (total mixed ration *ad libitum* (TMR) vs. separate (SEP) feeding of grass silage *ad libitum* and cereal concentrate) and housing (H) (cold vs. warm loose housing). The experiment was conducted between September and April. Average temperature was +2.7°C (min. –11.6°C, max. +14.9°C) in cold loose house and approximately +10°C in warm loose house. Blood was sampled 28 and 7 d prepartum and 1, 3, 5, 7, 14, 28 and 84 d postpartum.

Experiment 2: 24 Ayrshire cows were used in a continuous 2x2 factorial design. Experimental factors were dry period energy (E) level (normal (N) vs. high (H)) and glucogenic feed (G) (0 vs. 1 kg/d; G0 and G1). Glucogenic feed contained propylene glycol (120 g/kg), xylitol (4 g/kg), choline chloride 700 mg/kg and nicotine amide 1 g/kg. During weeks –8 to –4 before calving group H received 34 MJ/d more energy than N. From week –3 to calving, both groups received the same energy allowance. Glucogenic feed was fed starting 2 weeks before calving. After calving, cows received grass silage *ad libitum* plus fixed amount of cereal concentrate (max 15 kg/d). Blood was sampled 21, 7, 5, 3 and 1 d prepartum and 1, 3, 5, 7, 14, 21, 28 and 56 d postpartum.

Plasma leptin was determined with ovine specific RIA (Delavaud et al. 2000). In both experiments, cows were blocked according to their expected calving date. Results were analysed to test effects of experimental factors, their interactions and block.

**Table 1** Milk yield and feed intake, kg/d

| **Exp. 1** | Milk | DMI |
|---|---|---|
| Cold, TMR | 32.9 | 21.1 |
| Cold, SEP | 34.8 | 20.4 |
| Warm, TMR | 34.9 | 20.3 |
| Warm, SEP | 38.1 | 19.6 |
| S.e | 4.50 | 1.89 |
| **Exp. 2** | | |
| Normal, G0 | 34.7 | 19.5 |
| Normal, G1 | 36.7 | 18.7 |
| High, G0 | 36.1 | 17.3 |
| High, G1 | 39.4 | 18.8 |
| S.e | 3.61 | 1.56 |

**Results** No significant ($p<0.05$) differences between treatments in feed intake and milk yield were detected in neither of the experiments during lactation weeks 1 to 4 (Table 1). Average body condition scores at the time of calving in experiment 2 in groups N and H were 2.92 and 3.28 ($P<0.01$) and fat depths (including skin) near tuber ischii were 11.7 mm and 18.5 mm ($P<0.01$), respectively.

Plasma leptin was high in both experiments prepartum but decreased during the last week of pregnancy and the first week of lactation (nadir at d +7 *vs.* d +1 or d +3). After that, leptin started to increase slowly. In experiment 2, leptin was higher before calving (d –3: 5.2 vs. 3.1 ng/ml, $P<0.05$) and the decrease of leptin between d –3 and d +7 was larger (-3.1 vs. -1.4 ng/ml, $P<0.05$) in group H than in N.

**Table 2** Concentrations of plasma leptin, some hormones and metabolites

| **Experiment 1** | **Cold** | | **Warm** | | SEM | Sig [a] | |
|---|---|---|---|---|---|---|---|
| | **TMR** | **SEP** | **TMR** | **SEP** | | H | F |
| Leptin, ng/ml | 1.43 | 1.33 | 2.35 | 1.73 | 0.197 | ** | |
| NEFA, mmol/l | 0.43 | 0.54 | 0.41 | 0.40 | 0.062 | | |
| Insulin, µIU/ml | 5.9 | 7.0 | 6.8 | 7.5 | 1.07 | | |
| Glucagon, pg/ml | 125 | 153 | 101 | 116 | 13.5 | * | |
| Glucagon/Insulin | 23.0 | 29.3 | 15.8 | 19.0 | 4.01 | * | |
| **Experiment 2** | **Normal** | | **High** | | SEM | E | G |
| | **G0** | **G1** | **G0** | **G1** | | | |
| Leptin, ng/ml | 1.75 | 2.18 | 2.16 | 2.74 | 0.234 | | * |
| NEFA, mmol/l | 0.29 | 0.40 | 0.43 | 0.48 | 0.041 | * | |
| Insulin, µIU/ml | 5.4 | 5.4 | 5.2 | 6.3 | 0.82 | | |

[a] No significant interactions between experimental factors

In experiment 1, leptin was lower and glucagon and glucagon/insulin were higher ($P<0.05$) in cold loose housing during lactation weeks 1 to 4 (Table 2). In experiment 2, leptin was higher ($P<0.05$) with glucogenic feed and NEFA was lower ($P<0.05$) in N during lactation weeks 1 to 4.

Significant positive correlations were detected between leptin and insulin ($r>0.4$) and leptin and fat depth (experiment 2, $r>0.6$). Significant negative correlations were found between leptin and milk yield ($r>0.5$) and leptin and glucagon ($r>0.4$) during lactation weeks 2 to 4 in experiment 1. There were no significant correlations between leptin and total DMI during lactation weeks 1 to 4.

**Conclusions** Plasma leptin of dairy cows is high during late pregnancy, but decreases mainly during the last week before calving and first week of lactation. The decrease in leptin is more pronounced in fatter cows. Glucogenic feed and warm loose housing may increase plasma leptin during early lactation. However, changes in plasma leptin at that time are probably too small to influence feed intake regulation.

**Reference** Delavaud C., Bocquier F., Chilliard Y., Keisler D.H., Gertler A. and Kann, G. 2000. *J. Endocr.* 165: 519-526

# Serum leptin concentration is a poor predictor of body fat content in lactating dairy cows

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**Introduction** Blood concentrations of leptin have been associated, to varying degrees, with assorted measures of fat in mammalian species. In ruminants, 0.30, 0.35 and 0.37 of the variation in leptin levels in lambs (Blache *et al*., 2000), older (2-7 yr) multiparous ewes (Delavaud *et al*., 2000) and mature, late-lactation, dairy cows (Ehrhardt *et al*., 2000) was attributed to variation in fat as % of liveweight (LW), backfat thickness and body condition score (BCS) respectively while a strong correlation ($R^2$=0.83) between leptin and fat as % of empty body weight (EBW) was found for growing Holstein bull calves by Ehrhardt *et al*. (2000). The objective of the current study was to determine if leptin could be usefully related to fat in multiparous dairy cows differing widely across stages of lactation.

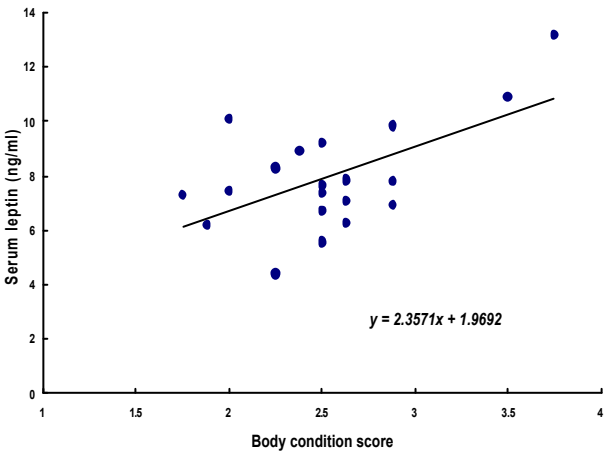
**Materials and methods** One hundred and eleven dairy cows of high and medium genetic merit, differing by stage of lactation (d15-d226), parity and liveweight (482-781kg), were selected from experiments at the Agricultural Research Institute and slaughtered on the same day at a commercial abattoir. Experimental diets were grass or grass silage based but differed in overall composition. All cows were fed up to the evening before slaughter. Blood samples were taken at slaughter and carcase and all non-carcase components were retained and analysed for lipid and other proximate constituents (Agnew *et al*., 2001). Serum leptin was determined using an improved, in-house, bovine/ovine RIA and concentrations were correlated against fat (as g/kg LW, g/kg EBW, g/kg carcase) and body condition score (BCS).

**Results** Fat in the LW, EBW and carcase was not correlated with serum leptin concentration at slaughter in cows from across the full lactation range in the study (15-226d) nor for cows between d15-120 or d140-226 of lactation (Table 1). Significant correlations between these fat measures and leptin were found in mid-lactation and were strongest for BCS in cows (*n*=21) between d130-170 of lactation (Fig 1) and similar to those seen by Blache *et al*. (2000) and Ehrhardt *et al*. (2000) for leptin *vs* fat in LW and leptin *vs* BCS in growing lambs and late lactation dairy cows respectively.

**Table 1.** Correlation coefficients ($R^2$) for serum leptin concentrations at slaughter and body fat in dairy cows

| Lactation range | *N* | Fat (% of LW) | Fat (% of EBW) | Carcase fat (%) | BCS |
|---|---|---|---|---|---|
| d15 – d226 | 111 | 0.004 (NS) | 0.003 (NS) | 0.004 (NS) | - |
| d15 – d120 | 43 | 0.004 (NS) | 0.0001 (NS) | 0.004 (NS) | - |
| d140 – d226 | 59 | 0.004 (NS) | 0.003 (NS) | 0.005 (NS) | - |
| d120 – d180 | 31 | 0.118 (P=0.029) | 0.110 (P=0.034) | 0.097 (P=0.044) | 0.165 (P=0.013) |
| d130 – d170 | 21 | 0.287 (P=0.006) | 0.246 (P=0.011) | 0.190 (P=0.024) | 0.341 (P=0.003) |

**Figure 1.** Relationship between serum leptin level and BCS in dairy cows (*n*=21) between d130-d170 of lactation




**Conclusion** Leptin concentrations were poorly related to measures of body fat in dairy cows across lactation and were not a useful predictor of fat in these animals. Fat mobilisation in early lactation may specifically distort leptin-fat relationships in dairy cows especially if, as we hypothesise (Wylie *et al*., 2002), leptin levels reflect lipogenesis rather than, or as well as, body fat content. The significant mass of mammary tissue might also affect the relationship between leptin levels and fat in lactating dairy cows compared with growing cattle.

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# The effect of grass or maize diet on plasma leptin and adipose tissue lipogenic enzyme activities in steers.

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**Introduction** Leptin is an important hormone for the control of food intake and body weight homeostasis in human and rodents. In ruminants, plasma leptin is positively related to body fatness and energy balance or feeding level (Chilliard *et al*., 2001) and linked to meat quality determinants such as marbling score (Minton *et al*., 1998). To our knowledge, no information is available on the effects of the nature of dietary forage on this parameter. The objective of the current work was to examine the effects of replacing maize silage with grass on plasma leptin as well as on the activities of five lipogenic enzymes in perirenal and inter-muscular adipose tissues (AT).

**Materials and methods** Twenty-four Charolais steers were allotted to two groups with two feeding regimes, "maize silage" (M) or "grass" (G). Grass-fed animals (n = 12) were offered fescue silage *ad libitum* completed with a commercial concentrate during winter and grazed on a rotational rye-grass pasture during the grazing season. Maize-fed animals (n = 12) were offered maize silage and soya-bean diluted with straw. After 24 months of feeding regime all the animals were killed at 30-32 months of age with a mean weight of 762-785 kg and the same body fat content (16% of fat in empty body weight). Jugular blood was sampled six times during the experiment period i.e. at the end and at the beginning of each turnout to grass and before slaughter to determine plasma leptin concentrations. Plasma leptin was measured by an ovine-specific leptin RIA which was shown to be efficient for bovine plasma (Delavaud *et al*., 2000). Samples of perirenal and inter-muscular AT were taken just after slaughter to assay the activities of lipogenic enzymes : fatty acid synthase (FAS), glucose-6-phosphate dehydrogenase (G6PDH), malic enzyme (ME), glycerol-3-phosphate dehydrogenase (G3PDH) and lipoprotein lipase (LPL) as described by Faulconnier *et al*. (2001).

**Results** Plasma leptin tended to be 27 % lower ($P < 0.06$) in the steers fed grass than in steers fed maize silage, but only in the older animals i. e. at the end of the experiment (interaction nature of forage x age of steers; $P < 0.03$) (Figure 1). Leptinemia was significantly higher in the older (30-32 months of age) than in younger (12-14 months of age) steers, whatever the feeding regimes ($P < 0.001$ and $P < 0.02$ for the M and the G groups, respectively). Moreover, LPL, G6PDH, ME, FAS and G3PDH activities were significantly ($P < 0.05$) lower by 49, 67, 56, 49, and 50 %, respectively, in perirenal AT and by 43, 67, 46, 34 and 28 %, respectively, in inter-muscular AT, in steers fed grass compared to maize silage (table 1).

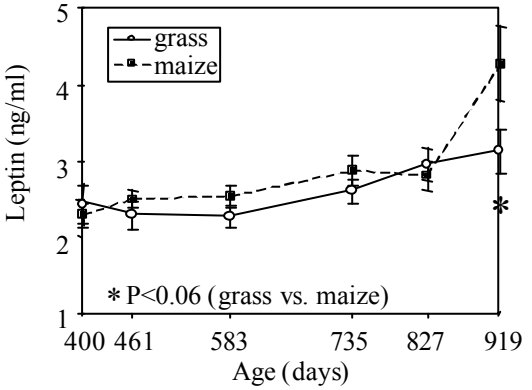


**Figure 1** Effect of nature of forage and age of the steers on plasma leptin concentration

**Table 1** Effect of nature of forage on AT cellularity and lipogenic enzyme activities (expressed as nmol/ mn/g of AT, Mean ± SD)

| | Perirenal AT | | Inter-muscular AT | |
|---|---|---|---|---|
| | Grass | Maize | Grass | Maize |
| Adipocyte volume (pl) | 1737±755 | 1402 ± 526 | 1419 ± 473 | 1183 ± 367 |
| LPL | 58 ± 6 | 115 ± 11 | 59 ± 7 | 102 ± 10 |
| G6PDH | 731 ± 60 | 2204 ± 170 | 590 ± 87 | 1774 ± 181 |
| ME | 74 ± 21 | 171 ± 16 | 182 ± 20 | 336 ± 26 |
| FAS | 36 ± 3 | 71 ± 10 | 36 ±3 | 55 ± 8 |
| G3PDH | 3722 ± 580 | 7397 ± 683 | 4146 ± 550 | 5753 ± 489 |

Effect of nature of forage was significant ($P<0.05$) for all variables except for adipocyte volume

**Conclusions** The results of the present study demonstrate that the ingestion of grass (compared to the ingestion of maize silage) decreased plasma leptin of the older steers and lipogenic enzyme activities in their perirenal and inter-muscular AT. This effect occurred despite the absence of any difference between the two diets in dry matter or energy intake level, growth rate and body composition, which were similar between the two groups.

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# The influence of dietary conjugated linoleic acid (CLA) on serum leptin concentration in lactating sows.

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**Introduction** Conjugated linoleic acid (CLA) is a mixture of geometrical and positional isomers of linoleic acid. Health-promoting properties of CLA, which include antioxidant, anti-obesity and anticarcinogenic activities, have been demonstrated in a wide range of animal models (Pariza *et al*., 2001). Recent studies indicated the CLA has a favorable effect on immune competence in nursery (Bassaganya-Riera *et al*., 2001) and weaned pigs (Corino *et al*., 2001). For this reason CLA may be useful in sow nutrition to increase CLA content in colostrum and milk (Bee, 2000). However CLA-fed animals displayed also significantly reduced body fat (Pariza *et al*., 2001) and this effect may be detrimental to reproductive efficiency in sows *per se* and for the effects on metabolic hormones as well. Moreover some CLA isomers has been reported to influence leptin gene expression (Houseknacht *et al*., 1998). The present study examined the effects of dietary supplement of CLA on serum leptin in lactating sows.

**Materials and methods** Sixteen Large White sows were divided into two experimental groups, control and treated. Starting on 15 days before parturition and continuing through lactation, treated animals were fed the control diet supplemented with 0.5% CLA preparation in free fatty acid form. The CLA mixture contained approximately 50 % of pure CLA isomers, 50% *cis 9, trans 11* isomer, and 50% *trans 10, cis 12* isomer (from certificate of analysis provided by the manufacturer). Body weight and Body Condition Score (BCS) were recorded before parturition and at weaning (21 d). Feed intake was measured daily. Blood samples were taken at 2, 10 and 20 days of lactation**.** Serum leptin concentrations, expressed as ng/mL human equivalent (HE), were determined with a commercially available radio-immunoassay procedure; the antibody was raised against human leptin and displayed 67% cross-reactivity to porcine leptin and detection limit of 1 ng/ml HE (Multi-Species Leptin RIA Kit, Linco Research Inc., MO, USA).

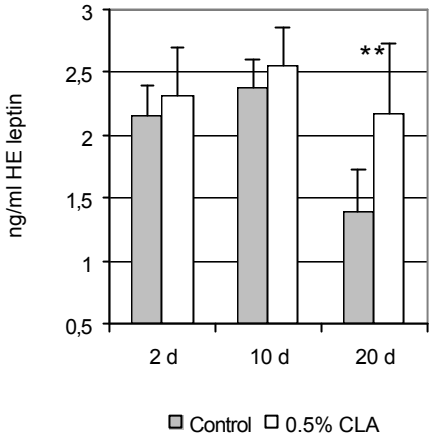


**Fig.**1 Serum leptin in lactating sows (mean ± s.d.)

**Results** CLA dietary supplementation did not affect body weight and BCS of the sows during lactation (Table 1). Average daily feed intake tended to be lower in CLA supplemented sows than in control, although no significant difference was observed. Average serum leptin concentration resulted significantly higher in sows fed CLA (2.37 *vs* 2.01 ng/ml HE, s.e.m=0.08) (P<0.01) (Figure 1). However it does not seem to be a relation between daily feed intake, body condition of sows during lactation and leptin release.

**Tab.1** - Feed intake, body weight, and body condition of sows (means ± s.d.)

| | Ctr | CLA | P |
|---|---|---|---|
| Sows, n | 8 | 8 | |
| Daily feed intake, kg/d | 4.54 ± 0.63 | .28 ± 0.70 | |
| Body weight, kg | | | ns |
| Before parturition* | 225 ± 31 | 233 ± 44 | |
| At weaning (21 d) | 201 ± 28 | 202 ± 42 | ns |
| BCS** | | | ns |
| Before parturition | 2.65 ± 0.32 | 2.68 ± 0.19 | |
| At weaning | 2.34 ± 0.42 | 2.36 ± 0.35 | ns |

* 7 d before parturition **Body Condition Score

**Conclusions** These results indicate that dietary CLA does not influence feed intake and body condition. in lactating sows. By these results it does not seem that circulating leptin in lactating sows may be related by body weight and body condition as established in humans and rodents. Further research could explain if the higher leptin level in CLA fed sows is due to changes in leptin gene expression or in leptin clearance as well.

**Acknowledgements** The research project was supported by the Italian Ministry for Universities and Scientific and Technological Research (Cofin.2000). The authors wish to express thanks to Pharmanutrients for providing the Conjugated linoleic acid (CLA) supplement for this project .

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# Leptin and its effect on glucose and insulin metabolism in pregnant and lactating goats

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**Introduction** Pregnancy and lactation are phases during which major adaptations in maternal metabolism are necessary to meet the requirements of foetal growth and of lactation. Leptin, an adipocyte derived hormone, involved in regulation of energy metabolism, has been implicated in the coordination of these adaptive processes. Similar to monogastric species, increased leptin blood concentrations are reported for sheep at mid-pregnancy when compared to prebreeding, late pregnancy or early lactation (Ehrhardt *et al*., 2001). In sheep, the changes of leptin concentrations showed no obvious relation with the ability of insulin to promote glucose utilisation (Ehrhardt *et al*., 2001). With the study presented herein, we aimed to elucidate whether exogenous leptin modulates insulin responsiveness and whether the responsiveness is dependent of the physiological status of the animal. Using specific clamp techniques, i.e. glucose infusion studies to quantify insulin secretion and resistance, we compared the effect of leptin application on glucose metabolism in pregnant versus lactating goats.

**Materials and methods** Eight primiparous White German Goats were used in a cross-over trial design, i.e. each animal served as its own control both during pregnancy and lactation. The goats were kept in loose housing with straw litter and had free access to hay and water. 0.2 kg concentrates (18% raw protein) were fed once per day and goat. Hyperglycaemic and euglycaemic-hyperinsulinaemic clamp experiments were performed during the last third of pregnancy and during early lactation. On the first day of the trial, i.e. between days 93 to 107 of pregnancy, both clamps were performed without any treatment to receive samples for the control group. During the 4 subsequent days, all goat received subcutaneous injections of recombinant ovine leptin (0,175 mg leptin/kg BW). On the fourth day of the injections, the clamp experiments were repeated. All clamps were done after an overnight fast. Catheters were inserted into both jugular veins, one for collecting blood samples and the other for glucose and/or insulin infusions. Two hours later, the hyperglycaemic clamp was performed: the blood glucose concentration of each goat was raised to about 40 mg/dl above the basal levels and was kept constant for two hours. Blood samples were collected in 5 min intervals, glucose concentrations were recorded (YSI1500G) and glucose infusion rates (GIR) were adjusted accordingly. Serum was obtained from the remaining samples stored at –30°C until assayed. After a 2 h pause, the euglycaemic-hyperinsulinaemic clamp was started in which the individual plasma glucose levels of the goats were maintained by infusing glucose during a permanent insulin infusion of 0,5µg insulin/kg BW x min. During lactation lambs were allowed to nurse at will. Starting at the 3$^{rd}$ day postpartum, all dams were again allocated to the clamp experiments, starting with control recordings and followed by leptin treatment as described for pregnant animals. Insulin was determined in serum as described by Bruckmann *et al*., 2000. Statistical comparisons were done with the individual mean GIR and insulin concentrations recorded when glucose concentrations were indeed clamped, i.e. 60 to 90 min after onset of the infusion when a plateau was reached. Normal distributed data were analysed by pairwise t-test and not normal distributed data by Wilcoxon`s test (SPSS program).

**Results** GIR are shown in table 1. During the control samplings without leptin treatment, GIR and insulin serum concentrations were not different between pregnancy and lactation neither during the hyperglycaemic nor during the euglycaemic-hyperinsulinaemic clamp. During pregnancy, GIR recorded during leptin application were lower compared to the preceding controls. Comparing pregnant and lactating goats during leptin treatment, about two-fold higher GIR were observed in lactating animals for both clamp types. In addition, lower insulin serum concentrations were observed in leptin treated pregnant dams compared to pregnant control goats during the hyperglycaemic clamp (1,39 ng /ml vs. 2,04 ng /ml, $p < 0.05$).

**Table 1:** GIR (mmol/kg BW x min; means ± s.e.m) in pregnant and in lactating goats treated with or without leptin

| | pregnancy-control | pregnancy-leptin | lactation-control | lactation-leptin |
|---|---|---|---|---|
| Hyperglycaemic | 13.87 ± 1.76$^{*}$ | 9.62 ± 1.32$^{*,\#}$ | 20.91 ± 2.66 | 23.60 ± 2.51$^{\#}$ |
| Euglycaemic-hyperinsulinaemic | 16.75 ± 2.14 | 15.62 ± 2.13$^{\#}$ | 20.16 ± 1.57 | 33.07 ± 4.27$^{\#}$ |

* designates differences between control and leptin treatment during pregnancy ($p < 0.05$)
\# designates differences between pregnant and lactating animals during leptin treatment ($p < 0.05$)

**Conclusions** Decreased GIR together with lower insulin serum levels in leptin treated versus control pregnant goats indicate that insulin sensitivity was increased by leptin treatment during pregnancy. In lactating goats, leptin application led to higher glucose turnover-rates than in pregnant animals. Our results indicate that the effectiveness of exogenous leptin to alter glucose metabolism is dependent of the physiological state of the animal, i.e. that pregnant and lactating dams respond divergently.

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# Influence of a nonforage diet on plasma leptin in dairy goats throughout lactation

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**Introduction** Leptin is a 16kDa peptide hormone mainly secreted by fat cells to regulate of food intake and energy homeostasis, and to signal the status of body energy stores to the brain (Houseknecht *et al*., 1998). In ruminant, reducing feedstuffs particle size increases DM intake, particularly if feedstuffs quality is poor, due to a shorter retention time of the particles in the rumen. By-products are included in the ration to supply energy and protein, but they have often a high content of fibre. The by-product fibre has different properties than forage NDF, being characterised by particles of small dimensions and a high density. The aim of this study was to compare the plasma level of leptin in lactating goats fed a traditional silage-based diet or a totally free forage diet, throughout lactation and during the pre and post-feeding state.

**Materials and methods** Eight second parity Saanen goats were assigned randomly to one of the two following dietary treatments: a silage-based forage diet with a forage to concentrate ratio of 55:45 (control, C) and a commercial nonforage diet (NF) containing 8.5% of whole cottonseed. Each group of four goats was fed its own assigned diet throughout the entire lactation. There was a great difference between the two diets for DM and some important chemical components (DM: 46 *vs* 91%; CP: 14.8 *vs* 21.4%; EE: 3.2 *vs* 5.6%; ADF: 17.8 *vs* 26.1%; ADL:2.1 *vs* 9.6%; NFC: 40.6 *vs* 28.8% on DM, for C and NF, respectively). The diets were offered for ad libitum intake twice daily. The trial was divided into three experimental periods of 8 d during early (40 DIM), mid (100 DIM) and late lactation (220 DIM). DMI was individually recorded daily during the three experimental periods. At the end of each period, blood samples were collected from each goat before the morning feeding and 4 h after feeding. Plasma leptin was determined by RIA (Linco Research Inc., St Charles, MO, USA). Data were statistically analysed using GLM Procedure of SAS (1996) with the repeated statement.

**Results** The lack of forages in NF diet did not affect body weight and milk production throughout lactation, but it significantly increased DMI (graph.); NF diet also reduced pre-feeding and post-feeding plasma leptin. These effects were more marked at early and mid lactation (table). Concerning the time-day effect, the post-feeding plasma leptin resulted higher than fasting leptin (3.22 *vs* 2.90 ng/ml, P=0.004).

**Table** Pre- and post-feeding plasma leptin levels (ng/ml) and daily dry matter intake (DMI) throughout lactation (Least squares means) (n=8).

| | Early | | Mid | | Late | | | Test of Effects ($P > F$) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Item | C | NF | C | NF | C | NF | SEM | Diet | Time-day | DIM |
| Pre-feeding leptin | 4.02 | 2.58 | 3.58 | 1.97 | 2.86 | 2.59 | 0.57 | 0.025 | 0.004 | 0.357 |
| Post-feeding leptin | 4.44 | 3.16 | 3.79 | 2.04 | 3.08 | 2.79 | 0.55 | | | |
| DMI, g/BW$^{0.75}$ | 135 | 164 | 126 | 147 | 91 | 95 | 10.2 | 0.022 | | 0.254 |

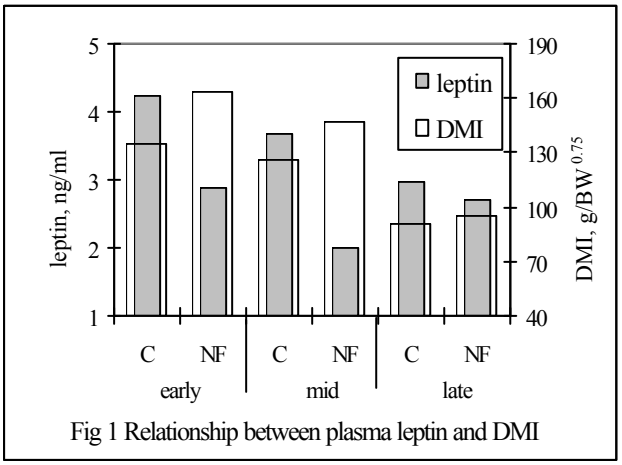

Fig 1 Relationship between plasma leptin and DMI

**Conclusions** The opposite trend in the pattern of diet effects on DMI and plasma leptin, both in pre- and post-feeding state, induces to retain that in goats throughout lactation the levels of leptin can be related to DM intake, as Barb (1999) proposed in other animal models.

Finally, it is interesting to note that also in lactating goats, as in other species, plasma leptin level increased during the post-feeding phase.

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# Leptin fully suppresses insulin secretion induced by acetylcholine and its effect is reversed by tolbutamide in *in vitro* perfused chicken pancreas

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**Introduction** The chicken leptin cDNA has been cloned and sequenced (AF012727, AF082500) (Taouis et al., 2001) and in this species, the leptin messenger has been found to be expressed not only in adipose tissue but also in the liver. In mammals, circulating leptin acts through specific receptors (Ob-Rb) located in the region of hypothalamus that regulates feeding behavior and energy expenditure; Ob-Rb have also been identified in the pancreatic ß-cells that produce insulin supporting evidence that leptin directly regulates insulin release (Kieffer et al. 2000). A direct effect of leptin on peripheral target tissues has not yet been demonstrated in chicken. The work was designed to study the effect of recombinant chicken leptin on acetylcholine-induced insulin secretion by isolated perfused chicken pancreas.

**Materials and methods** Four to six-week-old male broiler chickens (Shaver; 1,250-2,500 g) were used. Isolation and *in vitro* perfusion of the pancreaticoduodenal loop were performed as previously described (Rideau et al., 1986). After an equilibration period of 20 min (Krebs-Ringer buffer in the presence of 2.8 mM D-glucose), the perfusion medium was switched to include a mixture of D-glucose (14 mM) + acetylcholine bromide (Sigma, A6500, 1 µM). Twenty min after the beginning of the stimulation with acetycholine, recombinant chicken leptin was introduced through a side-arm syringe for 20 min. In another series of perfusion tolbutamide (Sigma, T0891, 100µM) was added through a second side-arm syringe 10 min after leptin, and perfused for 10 min. Insulin in perfusates collected at 1 min intervals in chilled tubes was measured by radioimmunoassay as described elsewhere (Rideau et al., 1986).

**Results** The time course of acetylcholine-induced insulin release and its inhibition by leptin are presented on fig.1 (mean of 3 perfusions for each series). Leptin rapidly (within 2 min) and significantly ($p<0.0001$) suppressed acetylcholine-induced insulin secretion with no change in volume outflow rate. Tolbutamide (100 µM) immediately and fully reversed the suppressive effect of leptin on acetylcholine-induced insulin release (fig.2 : mean ± SE, n= 3 perfusions).

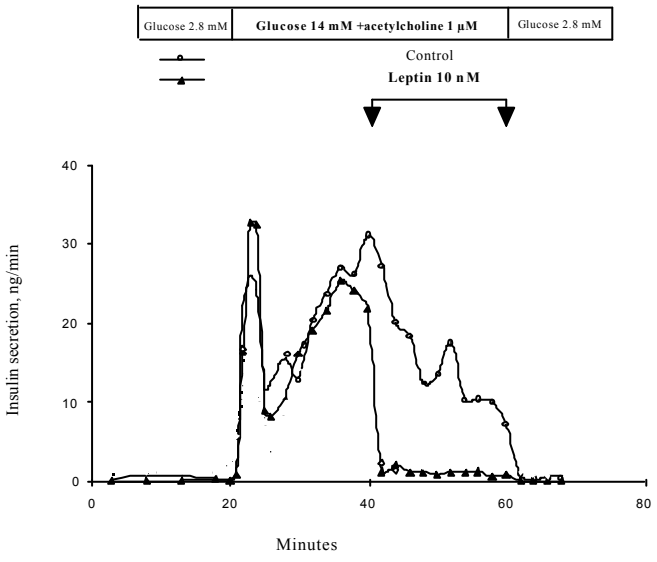


**Figure 1** Effect of leptin on acetylcholine-induced insulin release

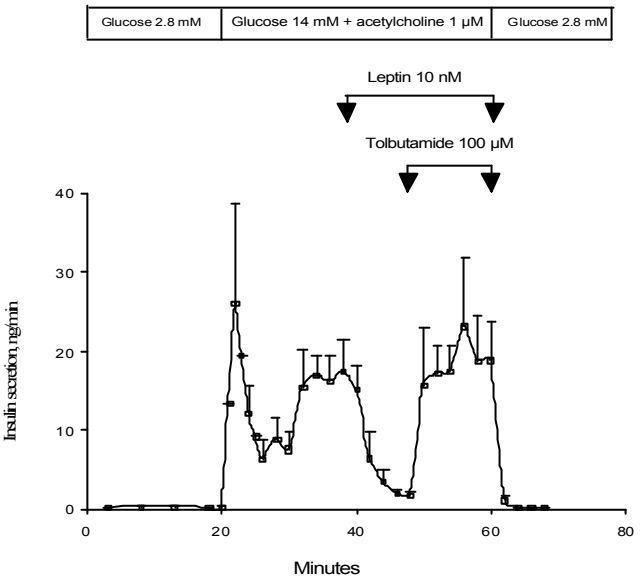


**Figure 2** Reversal of leptin effect by tolbutamide

**Conclusion** In conclusion, we show that recombinant chicken leptin (10 nM) exerts a sharp and immediate insulinostatic effect on acetylcholine-induced insulin secretion in isolated perfused pancreas of normal chicken. Inversion by tolbutamide of the leptin suppressive effect on insulin secretion suggests that $K_{ATP}$ channels may be a target for leptin to inhibit insulin secretion in normal chicken pancreas.

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# The effect of gender and physical parameters on plasma concentrations of leptin and thyroid hormones in the horse

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**Introduction** Leptin is a signalling factor involved in the regulation of body weight and is synthesised predominantly by adipocytes. In humans, there is a positive correlation between plasma concentration of leptin and body mass index ($kg/m^3$) and subcutaneous fat (Considine *et al.,* 1996; Lonnqvist *et al.,* 1995). I*n vitro* adipocytes obtained from women secrete more leptin than those of men (Casabeill *et al.*, 1998). Furthermore, testosterone inhibits the expression of the leptin gene in the rat (Wu-Peng *et al.,* 1999). The aim of this study was to examine whether gender, age and body conformation influenced plasma leptin and thyroid hormone concentrations in the horse.

**Materials and method** Pre-slaughter body weight and height were recorded in a random group of mares (n=5), geldings (castrated males: n=7) and stallions (n=3) destined for human consumption. Their age was estimated by dental examination. Immediately post-mortem, a jugular vein blood sample was collected into a heparinised tube, which was centrifuged at 2500 rpm for 10 minutes. Plasma was stored in liquid nitrogen until analysed for plasma concentrations of leptin and thyroid hormones using human ELISA (DRG Instruments) and RIA kits (ICN Pharmaceuticals Ltd), respectively. Statistical differences between groups were assessed using General Linear Model, Analysis of Variance. Regression analysis was used to determine whether plasma leptin concentration was related to body conformation and age.

**Results** There were no differences in body weight, height or age between the groups. Plasma concentrations of leptin, triiodothyronine ($T_3$) and thyroxine ($T_4$) were similar in mares and geldings but stallions exhibited lower ($P<0.05$) circulating levels of leptin (Table 1). Thyroid hormones were not related to age, body weight or height in mares and geldings. Plasma leptin concentration was related to age and body weight/height in mares and geldings as follows:

$$\text{Leptin concentration} = -52.7 + (1.06\ \text{Age}) + (2.10\ \text{weight/height}) \quad (R^2 = 87.3: P < 0.001)$$

A similar relationship was not observed in stallions.

**Table 1** Mean age, body weight, height, and plasma concentrations of $T_3$, $T_4$ and leptin

| | Age (years) | Weight (kg) | Height (cm) | Leptin (ng/ml) | $T_3$ (ng/ml) | $T_4$ (ng/ml) |
|---|---|---|---|---|---|---|
| Mare (n=5) | 10.8±2.3 | 477±24 | 164±7 | 26.1 ±3.4 | 3.4 ±0.2 | 25.6 ±1.6 |
| Gelding (n=7) | 8.1±1.6 | 572±22 | 169±4 | 24.2 ±3.2 | 3.1 ±0.2 | 28.3 ±2.5 |
| Stallion (n=3) | 9.3±1.8 | 505±9 | 167±5 | 15.6 ±1.7* | – | – |

Values are mean ± SEM. *P<0.05.

**Conclusions** Similar age, body weight/height and gender dependent relationships are present in horses that have already been observed in man, rats and other animals. Furthermore, it would appear that castration reduces the inhibitory effect of testosterone on leptin secretion with the result that leptin concentrations in geldings are similar to those observed in mares.

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# Influence of food intake timing on daily variations of leptin and other metabolic variables in nzw rabbits

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**Introduction** The 16kDa peptide hormone leptin is an adipose tissue-derived regulator of food intake and energy homeostasis, and a signal of the status of body energy stores to the brain. Plasma levels of leptin reflect body fat mass in humans, rodents and ruminants (Houseknecht *et al.*, 1998; Delavaud *et al.*, 2000). The aim of this study was to investigate circadian rhythms of plasma leptin and other metabolic variables in rabbits, to assess the influence of the timing of food intake and to investigate the relationship between leptin and lipid metabolites.

**Materials and methods** Forty-eight male New Zealand White rabbits, 2.5 kg (SEM ± 13 g), housed individually with light from 08.00 to 20.00 h, and fed a commercial diet, were divided into two age and weight matched groups. One had access to feed 20.00-08.00 h (group N) and the other 08.00-20.00 h (group D). Individual feed intake was monitored every 4 h. On the 16th day, blood samples from different groups of four animals were taken every 4 h over the ensuing 24 h, after recording rectal temperature. Leptin (Linco Research Inc., St Charles, MO, USA), corticosterone (ICN Pharmaceuticals Inc., Costa Mesa, CA, USA) and somatostatin (Hilsted L. et Holst J.J. 1982. *Reg. Peptides* **4**: 13-31) were determined by RIA on plasma (Na-EDTA as anticoagulant and aprotinin as protease inhibiter). Plasma metabolites were determined by enzymatic-colorimetric methods (Boehringer Mannheim GmbH, Mannheim, D). The data were analysed by periodic regression (Bingham C. et al. 1982. *Chronobiol*. **9**: 397-439) to reveal circadian periodicity and to estimate parameters for best-fit cosine curves for the data of each group. The F test was used to test the zero amplitude hypothesis and the validity of the cosine model at the probability level of 0.05. The cosine function was $y = y + A\cos(\omega t + \varphi)$, in which y is the mean of the physiological variable, A the amplitude, $\omega$ the angular velocity (here 360°/24h), t is the time (independent variable; hours after the reference time 00.00 h) and $\varphi$ is the acrophase (time, in degrees from reference point, at which the cosine function attains its maximum).

**Results** Body weights and quantities of food ingested did not differ significantly between the two groups over the experimental period. The other variables showed significant 24 h sinusoidal variations in both groups, in all cases similar to those illustrated in Fig. 1 for leptin. Since the variations in group N were shifted by about 12 hours relative to those in group D, we pooled the data from the two groups, using as time reference (00.00 h) the onset of the feeding period (Table 1). On both feeding schedules, leptin, somatostatin and urea levels and body temperature peaked about 10 hours after the start of feeding; triglyceride and phospholipid levels peaked soon after feeding stopped; NEFA and corticosterone levels peaked at the end of the fasting period. Leptin levels correlate inversely with NEFA and corticosterone and directly with triglyceride, phospholipid and somatostatin (Table 2).

**Table 1** Parameters of circadian rhythms of variables analysed by periodic regression in rabbits fed 12 hours a day. Data are synchronized to onset of feeding period

| VARIABLE | MEAN | SEM | A | % RHYTHM | ACROPHASE (h) | P |
|---|---|---|---|---|---|---|
| Body temperature °C | 39.71 | 0.05 | 0.27 | 23.3 | 8:19 | <0.001 |
| Leptin µg/l | 3.03 | 0.08 | 0.46 | 30.8 | 11:08 | <0.001 |
| Somatostatin pmol/l | 15.40 | 0.16 | 5.24 | 18.3 | 9:55 | 0.01 |
| Corticosterone µg/l | 25.36 | 0.82 | 7.34 | 47.1 | 22:47 | <0.001 |
| NEFA µmol/l | 161.2 | 8.88 | 44.3 | 31.7 | 21:16 | <0.001 |
| Triglyceride mmol/l | 1.05 | 0.06 | 0.19 | 13.8 | 13:45 | 0.03 |
| Phospholipid mmol/l | 0.93 | 0.03 | 0.10 | 11.5 | 13:58 | 0.06 |
| Urea mmol/l | 5.75 | 0.15 | 1.34 | 46.2 | 7:58 | <0.001 |
| Glucose mmol/l | 10.9 | 0.30 | 1.04 | 21.1 | 16:46 | <0.01 |

P= Likelihood of finding an F value the same as that observed if there were no variations in circadian patterns

**Table 2** Correlations of leptin with:

| | r | P |
|---|---|---|
| Body temperature | 0.499 | <0.05 |
| Somatostatin | 0.878 | <0.01 |
| Corticosterone | -0.922 | <0.01 |
| NEFA | -0.715 | <0.05 |
| Triglyceride | 0.616 | <0.05 |
| Phospholipid | 0.639 | <0.05 |
| Glucose | 0.154 | ns |
| Urea | 0.625 | <0.05 |

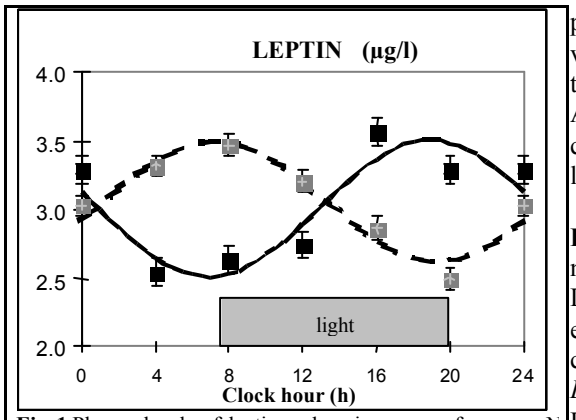


**Fig 1** Plasma levels of leptin and cosine curves for group N (dashed line, grey dots) and group D (black line, black dots)

**Conclusions** The data of this study show clearly that the time of administration of food is a potent synchronizer of the circadian patterns of leptin and the other parameters investigated. The diurnal variations in leptin therefore depend on the "nutritional status" of the animal, rather than light cues, as Schoeller *et al.* (1997) and Ahima *et al.* (1996) also inferred in humans and mice. Leptin correlations with the variables investigated indicate that in rabbits leptin is an index of the lipid metabolism.

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## Replacement of water by liquid whey and its influence on performance of Holstein steers

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**Introduction** Liquid whey as a by-product of dairy industry is produced in a large quantity in Iran. Feeding fresh whey to livestock is an economical and applicable method of it's utilization in the country. This experiment was designed in order to evaluate the effects of using liquid whey instead of drinking water in Holstein steers.

**Material and Methods** Twelve Holstein steers with average initial body weight of 140±20 Kg were stratified based on body weight into three groups and randomely assigned to the dietary treatments in a completely randomized design with four replicates. The dietary treatments were: ?) Free access to drinking water (control) II) Free access to drinking water and liquid whey III) Free access to liquid whey. Diet was formulated based on NRC (1989) recommendations. All animals received alfalfa hay at %0.7 of body weight (DM Basis) and fed concentrate *ad libitum.* The concentrate mixture contained following ingredients: barley %37.5, cottonseed meal %25.2, dry beet pulp %18.2, wheat bran %12, ground corn grain %5.2, limeston %1, salt %0.4 mineral premix %0.25 and vitamin premix %0.25. The feeding period was 98 days including 13 days adaptation. The blood samples were collected from jugular vein 3 hrs post morning feeding in the beginning, mid and end of the feeding period and their plasma were separated for determination of plasma urea nitrogen (PUN) by centrifuging at 3000 $^{RPM}$ for 10 minutes. Rumen liqour was sampled by stomach tube and suction pump for determination of its pH and ammonia nitrogen content ($NH_3$-N). Feed and feces analyses were conducted according to the methods of AOAC (1984). Acid insoluble ash (AIA) was used as internal marker for measuring apparent digestibility (Van Keulen and Young 1977). The data were analyzed using ANOVA procedure ( SAS 1996).

**Results** Voluntary intakes of water, whey, concentrate and alfalfa are given in table 1. Total liquid intake by the steers in treatment II increased by 36.8 percent. Concentrate consumption in treatments II and III reduced by 50 percent in comparison with the control ($p<0.01$).

Table 1- Average water, whey, alfalfa and concentrate intake by the experimental steers (Kg/d)

| | Treatment | | | |
|---|---|---|---|---|
| | I | II | III | SE |
| **Water** | **36.55** | **5.7** | **-** | **4.47** |
| **Whey** | **-** | **44.29** | **48.48** | **5.67** |
| **Total liquid intake** | **36.55** | **49.99** | **48.48** | **5.38** |
| **Alfalfa (DM)** | **1.34** | **1.13** | **1.11** | **0.106** |
| **Concentrate (DM)** | **4.99** | **2.62** | **2.64** | **0.34** |
| **Whey (DM)** | **-** | **2.40** | **2.63** | **0.307** |
| **Total DMI** | **6.30** | **6.15** | **6.37** | **0.25** |

Table 2- Apparent nutrient digestibilities (g/g)

| | Treatment | | | |
|---|---|---|---|---|
| | I | II | III | SE |
| **DM** | **0.797** | **0.755** | **0.763** | **0.047** |
| **OM** | **0.809** | **0.765** | **0.774** | **0.014** |
| **CP** | **0.770** | **0.788** | **0.806** | **0.046** |
| **CF** | **0.535** | **0.378** | **0.342** | **0.088** |
| **NDF** | **0.682** | **0.415** | **0.413** | **0.106** |
| **ADF** | **0.462** | **0.337** | **0.238** | **0.063** |

Apparent digestibilities of NDF in treatments II and III were lower than control (Table 2). The ADF digestibility in treatment III was lower than control treatment. DM, OM, CP and CF digestibilities were not affected by treatments. Ammonia nitrogen ($NH_3$-N) of rumen liquid in treatments II and III was lower than treatment I in first sampling (13.36, 6.08 and 7.59 mg/dl in treatments I, II and III respectively SE = 1.93). Plasma urea nitrogen (PUN) in treatments II and III was higher than the control in first sampling (17.41, 22.33 and 25.15 mg/dl in treatments I, II and III respectively SE = 2.51). Average daily weight gain (ADG) and feed conversion ratio (FCR) (Kg DMI/Kg gain) were not different among the treatments (Table 3). Rumen and blood pH were not affected by treatments.

Table 3- Average daily gain and feed conversion ratio

| | Treatment | | | |
|---|---|---|---|---|
| | I | II | III | SE |
| **Average daily gain (Kg/d)** | **1.318** | **1.423** | **1.415** | **0.106** |
| **Feed conversion ratio (Kg/Kg)** | **4.855** | **4.355** | **4.560** | **0.360** |

**Conclusion** The results of this experiment indicated that liquid whey can be successfully fed to Holstein steers without adverse effects onaverage daily gain and feed conversion ratio.

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# The use of production data of feeding studies in lactating dairy cows to validate energy feeding systems

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**Introduction** There is little information available in the literature on the validation of the currently adopted energy feeding systems developed from calorimetric data, using data obtained in production studies. The objective of the present study was to use production data from feeding studies to validate some metabolisable energy (ME) systems (AFRC, 1990 and 1993; SCA, 1990) and net energy (NE) systems (Van Es, 1978, INRA, 1989; NRC, 2001).

**Material and methods** The production data used in the present study were derived from 838 lactating dairy cows drawn from 12 long term feeding trials (at least eight weeks/period) across Northern and Southern Ireland. The animals were offered mixed diets of concentrates and silages of grass (n=33) and maize (n=5) *ad libitum.* The cows were of various genetic merits and had a range of lactation numbers (1-9). The ME concentrations in the mixed diets were either measured directly in calorimetric studies or estimated from the *in vivo* digestible organic matter in total dry matter (DM) (AFRC, 1993). The ME requirement for pregnancy estimated for late pregnant cows was subtracted from total ME intake. The daily energy output in milk was calculated and live weight change (LWC) was estimated from the linear regression of live weight against the recording date (weeks). The validation was carried out using linear regression and mean-square prediction error (MSPE).

**Results** The mixed diets offered contained proportionately 0.30 to 0.87 forage in the total diet (DM basis). The milk yields varied from 7.7 to 48.9 kg/d and total ME intake from 91 to 338 MJ/d. Live weight was significantly related to recording date for all cows ($P<0.05$ or less) and LWC ranged from –1.23 to 1.73 kg/d. There was a significant relationship between actual energy intake and predicted energy requirement ($P<0.001$) ($R^2$ = 0.69 to 0.74) for each of the energy feeding systems. AFRC (1990), Van Es (1978) and NRC (2001) each under-predicted total energy requirements by proportionately 0.06, 0.04 and 0.03 respectively, while SCA (1990) and INRA (1989) performed relatively well. AFRC (1990) produced a relatively large error in the bias (actual ME intake – predicted ME requirement) over the total MSPE, and a relatively smaller error in random than other systems. The error in random over the total MSPE for Van Es (1978) and NRC (2001) was close to SCA (1990) and INRA (1989). However an addition of proportionately 0.05 to the total predicted ME requirement of AFRC (1990), as suggested in AFRC (1993) for the practical use in UK, improved the prediction accuracy to be similar to other systems. Nevertheless, all the systems had a poor prediction on LWC and the relationship between actual and predicted LWC was very poor ($R^2$ = 0.04-0.06), although significant ($P<0.001$). Each system produced a large error of line over total MSPE (0.49 to 0.64), indicating a large variation between actual and predicted LWC existed among individual cows.

**Table 1.** The accuracy of prediction of energy requirement or LWC using different feeding systems (n=838)

| | Energy prediction (MJ/kg) | | | | | | LWC prediction (kg/d) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Energy | | | Proportion of MSPE | | | LWC | | | Proportion of MSPE | | |
| | Act. | Pred. | MPE | Bias | Line | Random | Act. | Pred. | MPE | Bias | Line | Random |
| AFRC (1990) | 192 | 181 | 0.13 | 0.18 | 0.09 | 0.73 | 0.23 | 0.55 | 3.14 | 0.21 | 0.49 | 0.30 |
| AFRC (1993) | 192 | 189 | 0.12 | 0.02 | 0.12 | 0.86 | 0.23 | 0.64 | 3.64 | 0.25 | 0.53 | 0.22 |
| SCA (1990) | 192 | 193 | 0.11 | 0.00 | 0.17 | 0.83 | 0.23 | 0.23 | 2.92 | 0.00 | 0.64 | 0.36 |
| Van Es (1978) | 118 | 114 | 0.12 | 0.09 | 0.11 | 0.80 | 0.23 | 0.45 | 3.14 | 0.10 | 0.59 | 0.31 |
| INRA (1989) | 118 | 119 | 0.13 | 0.00 | 0.19 | 0.81 | 0.23 | 0.23 | 2.50 | 0.00 | 0.52 | 0.48 |
| NRC (2001) | 122 | 119 | 0.12 | 0.05 | 0.12 | 0.84 | 0.23 | 0.32 | 2.65 | 0.02 | 0.55 | 0.43 |

**Conclusion** AFRC (1993) and other foreign systems can predict relatively well total energy requirement, while all systems tested had a poor prediction of LWC. However, an addition of proportionately 0.05 to total ME requirement of AFRC (1990) as suggested in AFRC (1993) is not justified, because there is ample evidence indicating that the under-prediction by AFRC (1990) is likely from maintenance, but not from production.

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# Maternal nitrogen balance of dairy cows during late gestation

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**Introduction** Previous results have shown that during late gestation even under conditions of live weight (LW) gain, maternal body protein can be in negative balance due to the highly demanding gravid uterus and udder. It has also been claimed that current feeding standards underpredict dry cow nitrogen (N) requirement. Considering that it is not possible to measure maternal body N status independently of the requirements of the conceptus and the udder, estimation of conceptus and udder N requirements by mathematical models can help to predict maternal N requirement. The aim of this study was to assess cow N requirement during late gestation by predicting maternal N balance through a mathematical model. Previous results related with this study were presented in Jaurena *et al*, (2001).

**Material and methods** The parameters necessary to construct the mathematical model were estimated in a N balance performed at 3 weeks before calving, involving measurement of feed dry matter intake (DMI), refusals and total collection of faeces and urine. The experimental diets were based on ryegrass silage alone or supplemented with 50 g prairie meal per kg dry matter (DM) and 100 g of Megalac™ per kg DM in a factorial arrangement. Rations varied in N×6.25 (CP) content between 151 and 166 g/kg DM, and metabolisable energy between 10.4 and 11.1 MJ /kg DM. Each treatment was individually offered *ad libitum* for 6 weeks prepartum to four cows grouped according to calving date. Scurf N losses were predicted (AFRC, 1993), metabolic faecal N (MFN) and N true digestibility (NTDig) were estimated by regression analysis of N digested on diet N concentration (both expressed on a per kg DM basis). Endogenous urinary N (EUN) was estimated as the regression of N retained ($N_{Ret}$) on N intake ($N_{Int}$; both expressed on LW $kg^{0.75}$ basis). Predicted cows' N balances by this model were compared with the actual cows' N balances at three weeks before calving, yielding the following adjusting equation: actual Nbal (g/d) = –17.1 + 1.2 × predicted Nbal (g/d); $r^2 = 0.60$). Based on the parameters estimated with the balance technique, maternal N balance (MNB) was predicted for the last six weeks of gestation, assuming the dry matter intake (DMI) prediction provided by NRC (2001) and two CP contents (120 and 140 g/kg DM) based on the following equations:

$$MNB\ (g/d) = 1.2 \times \{DMI \times [N] - (N_{Fc} + N_{Ur})\} - 17.1 - N_{GU} - N_{Udder};$$
$$N_{Fc}\ (g/d) = N_{Int} \times (1 - N_{TDig}) + MFN \times DMI;$$
$$N_{Ur}\ (g/d) = \{N_{Int}/LW^{0.75} \times ß_{Ur/Int} - EUN/1000\} \times LW^{0.75}$$

Where $N_{Fc}$, faecal N losses; $N_{Ur}$, urinary N losses; $N_{GU}$, N retained in the gravid uterus (AFRC, 1993); $N_{Udder}$, N retained in the udder (estimated as 0.48 and 7.4 g N/day for less and more than 21 days to calving respectively, and assuming 100 g CP/kg content in parenchyma tissue (Capuco *et al.*, 1997; NRC, 2001); $ß_{Ur/Int}$, regression coefficient of $N_{Ur}$ on $N_{Int}$ expressed on $LW^{0.75}$ basis. Dry matter intake was estimated assuming a cow of 625 kg LW at 220 days of gestation, gaining 500 g/d of LW and consuming a diet of 0.72 (g/g) of organic matter digestibility.

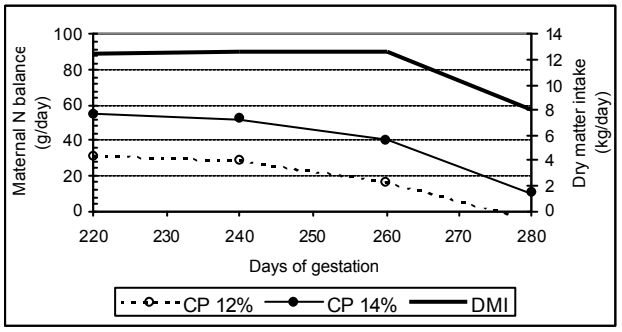


Figure 1. Maternal N balance and estimated dry matter intake (DMI) during the dry period.

**Results** Estimation of MFN (6.62 g/kg DMI), EUN (133 $g/kg^{0.75}$LW) and $N_{TDig}$ (0.96 g/g) were similar to other values reported in the literature, and MNB was positive for 120 and 140 g CP /kg DM contents during the last 60 days of gestation. However, as this prediction did not include requirements for colostrum synthesis, it is possible that dairy cows fed with similar diets at 120 g/kg DM CP content could suffer a transient N deficit during the last few days of gestation. It must also be stated that as first and second calvers have a growth N requirement and lower DMI, their diets' minimum CP content will be higher.

**Conclusion** During late gestation, mature dry cows should be offered diets with no less than 120 g CP/kg DM to avoid mobilisation of maternal body N to supply the requirement of the conceptus and the udder development.

**Acknowledgements** This work was funded by the Ministry of Agriculture, Fisheries and Food. G. Jaurena is grateful for scholarships provided by the British Council and Fundación Antorchas (Argentina).

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# Application of a dynamic mechanistic model of small intestinal starch digestion in the dairy cow

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**Introduction** The high contribution of postruminal starch digestion (>50%) to total tract starch digestion on certain energy dense diets (Mills et al. 1999) demands that limitations to small intestinal starch digestion are identified. Therefore, a dynamic mechanistic model of the small intestine was constructed and evaluated against published experimental data for abomasal carbohydrate infusions in the dairy cow. The mechanistic structure of the model allowed the current biological knowledge to be integrated into a system capable of identifying restrictions to dietary energy recovery from postruminal starch delivery.

**Materials and Methods** The model is based on the principles advanced by Mills et al. (1999). The seven state variables represent starch, oligosaccharide, glucose and pancreatic amylase in the intestinal lumen, oligosaccharide and glucose at the unstirred water layer (UWL), and the intracellular glucose of the enterocyte. Enzymic degradation of starch is a two-stage process involving luminal pancreatic amylase and oligosaccharidase on the brush border of the enterocyte confined within the UWL. $Na^+$ dependent glucose transport (SGLT1) into the enterocyte is represented along with a kinetically asymmetrical facilitative GLUT2 transport system on the basolateral membrane. The small intestine is subdivided into three main sections representing the duodenum, jejunum and ileum for parameterisation. Further sub-sections are defined between which there is a continual flow of digesta represented as a fractional rate. The fluxes are described by either mass action (e.g. diffusion) or Michaelis-Menten (e.g. enzyme activities) kinetics. Parameterisation of these equations was performed with appropriate data taken from the literature concerning in vivo and in vitro studies using both ruminant and non-ruminant animals. The differential equations for each state variable are solved with a Runge Kutta forth order fixed step algorithm using the Advanced Continuous Simulation Language.

**Results** The model predicted non-structural carbohydrate disappearance for cattle unadapted to duodenal infusion (Kreikemeier et al. 1991 & 1995, Krehbiel et al. 1996) with an $r^2 = 0.92$ and a root mean square prediction error (rootMSPE) of 25.4%. Simulation of glucose disappearance for mature Holstein heifers adapted to various levels of duodenal glucose infusion (Cant et al. 1999) yielded an $r^2 = 0.81$ and a rootMSPE of 38.6%. Behavioural analysis identified the limitations for small intestinal starch digestion efficiency at high levels of duodenal starch appearance. There was a spatial asynchrony between enzymic digestion in the proximal small intestine and glucose transport capacity towards the terminal ileum. Therefore the potential for starch to be degraded to glucose but remain unutilised within the small intestine existed for diets where postruminal starch delivery was high.

**Conclusions** Limitations to individual metabolic processes, particularly to starch digestion in the proximal section of the intestine, can create asynchrony between starch degradation and glucose uptake capacity. The model indicated that there were a series of rate limiting steps in small intestinal starch metabolism including pancreatic amylase secretion, oligosaccharidase activity, and glucose uptake via SGLT1.

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# Calcium metabolism in Saanen goats- a kinetic model

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**Introduction** Research in Brazil has been carried out to study mineral metabolism in sheep and cattle, especially phosphorus, by using the isotope dilution technique (Vitti et al., 2000). However there is very little information on calcium metabolism in goats. The proposal of the present study is to measure the intensity of the different processes that occur in goats fed different amounts of calcium, by using radioactive calcium, and to propose a model of Ca metabolism.

**Material and methods** During a 28 days period, 9 Saanen goats received rations containing three levels of Ca ($T_1$=0.06, $T_2$=0.17 and $T_3$= 0.30%DM) (Table 1). Each animal was intravenously injected with 7.4.MBq of radio-calcium. Blood samples, feces and urine were taken at 24-h intervals. Total calcium and radioactivity in all the samples were measured. After the end of collection period tissues samples were collected (liver, heart, kidney, muscles and 12$^{th}$ rib) for analysis.

**Statistical Analyses** Experimentally measured (model inputs) and model output was statistically analyzed. The data used in the analysis were from 9 animals, 3 from each level of Ca intake. A comparison of means from each category and regression analysis was carried out using the GLMP ( SAS, 1991) with the sources of variation being the goats in each treatment and level of Ca inclusion. Treatment means were assessed for significant differences at $P$ <0.05.

**Results** Figure 1 shows the schematic representation of the model of calcium metabolism. The average Ca intake was 22.82, 53.40 and 93.3 mg/kg BW/day respectively for $T_1$, $T_2$ and $T_3$. Calcium intake ($F_{10}$) affected the total Ca excreted in faeces ($F_{01}$) $F_{01}$=0.228+3.46$F_{10}$ (n=9; $r^2$=0.79; P<0.001). The endogenous faecal loss ($F_{e01}$) was 0.13, 0.27 and 0.31 g Ca/d for treatments $T_1$, $T_2$ and $T_3$, respectively (P>0.05). Truly absorbed Ca, ($F_{d21,}$ where $F_{d21} = F_{10} - (F_{01} - F_{e01})$), was 0.28, 0.71 and 1.55 g Ca/d for treatments $T_1$, $T_2$ and $T_3$, respectively (P<0.01). Ca was absorbed from the $T_1$ diet at 47% efficiency (true absorption/intake), and absorption from $T_2$ and $T_3$ diets was at 59 and 62% efficiency, respectively. The lower efficiency of Ca absorption observed for $T_1$ may reflect the low availability of Ca, mainly present in the organic form. There was a significant linear relationship between the truly absorbed Ca and Ca intake: $F_{d21}$ = -0.09 + 0.653 $F_{10}$ (n=9; $r^2$=0.97; P<0.01).

**Table 1**. Feed ingredients of the diet (g/kg DM) fed to goats

| Feed | $T_1$ | $T_2$ | $T_3$ |
|---|---|---|---|
| Corn whole plant | 288.0 | 279.0 | 282.5 |
| Corn cobs and straw | 295.0 | 295.0 | 288.0 |
| Corn grains | 402.0 | 408.0 | 408.0 |
| Limestone | 0 | 3.0 | 6.5 |
| Salt mineral | 15.0 | 15.0 | 15.0 |

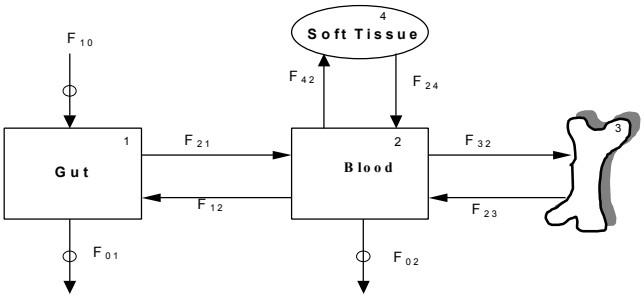


**Figure 1** Schematic representation of calcium metabolism model

[a]Composition per kg DM: 8.73 mg Fe; 7.64 mg Cu; 44.36 mg Mn;58.73 mg Zn; 0.1 mg Co; 0.2 mg I e 0.03 mg Se.

Retention of Ca was 0.08, 0.39 and 1.20 g/d for animals fed $T_1$, $T_2$ and $T_3$ respectively, and it increased significantly with increased Ca intake ($F_{10}$) and absorption ($F_{d21}$) (P<0.01). The increased total body retention of Ca reflected an increase in the skeletal retention ($F_{32}$-$F_{23}$= 0.19; 0.46 and 1.33 g Ca/d for $T_1$, $T_2$ and $T_3$, respectively), which was probably brought by a decrease in the rate of bone re-sorption. Calcium flow from the digestive tract into the blood pool ($F_{21}$) increased with Ca intake ($P$<0.001) ($F_{21}$=0.014+0.825$F_{10}$ ($r^2$=0.91, P<0.01). The Ca flux from blood to soft tissue ($F_{42}$) was higher in goats fed diet $T_3$ ($P$<0.05). The relationship between Ca from blood to soft tissues ($F_{42}$) and Ca intake ($F_{10}$) was $F_{42}$=0.00006+0.0008$F_{10}$ ($r^2$= 0.68, P<0.01). Bone re-sorption ($F_{23}$) was not influenced by Ca intake.

**Conclusions** The kinetics model could be used to illustrate the different processes that occur in goats fed various Ca levels and it showed that Ca intake influenced Ca absorption, retention and excretion.

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# Application of a dynamic nitrogen model to reduce nutrient pollution by dairy cows

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**Introduction** Agriculture is one of the major sources of nitrogen (N) pollution. Dairy production causes losses of N in faeces and urine that contribute to environmental pollution with an estimated annual output of 320 kt N and 80 kt ammonia in the U.K. alone. Therefore, improving N utilization in dairy cows and especially reducing N output in excreta is desirable to reduce environmental N pollution, particularly as ammonia. Mathematical models have been used to predict potential N excretion from dairy cows. However, these models generally are empirical in nature, hence not process based and therefore there is a need to develop a model that can describe biological processes in the animal. The objective of the present study was to develop a dynamic N model to predict the amount and form of N excreted by dairy herds and seek to make appropriate recommendations that will reduce N excretion from dairy cows.

**Materials and methods** Data from five experiments conducted at the University of Reading were used to develop the model. The trials were conducted with 30 multiparous Holstein/Friesian dairy cows in early or mid lactation fed 30 different diet types consisting of 10 grass silages and 6 concentrates.

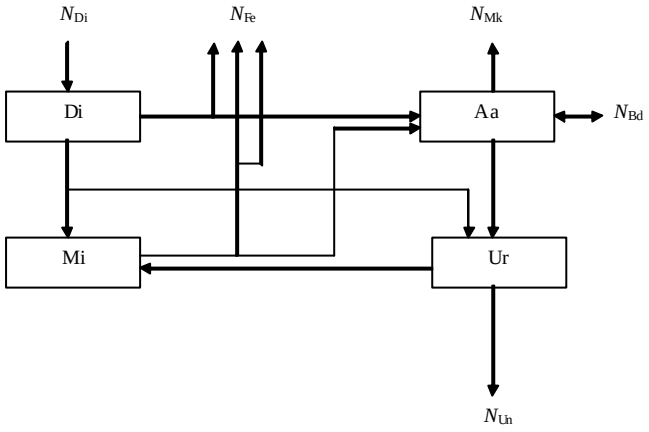


**Figure 1** Schematic representation of the dynamic model of N metabolism in a lactating dairy cow (Kebreab et al. 2001)

The model contains four pools, and arrows represent inputs and outputs to and from the pools (Fig. 1). The abbreviations represent; dietary N (Di), amino acid N (Aa), urea N (Ur), and microbial N (Mi). The subscripts denote faecal (Fe), milk (Mk), body (Bd) and urinary (Un) N. For simplicity, ammonia and urea N are aggregated, with N flowing out of the rumen in the form of ammonia and being transformed to urea in the liver. The model was programmed in the Advanced Continuous Simulation Language and a fourth-order Runge-Kutta method was used for numerical integration. The model was run until a steady-state was achieved for each level of N intake.

**Results** The model was used to estimate N pollution from dairy cows and simulate scenarios to quantify effects of nutrition on N excretion. Simulation using the model showed that the relationship between N intake and faecal N was linear up to 420 gN/d; after which another linear but flatter relationship was observed. A similar response was also observed for milk N output. However, urinary N output had a curvilinear response to increased levels of N intake. Increases in N without a matching increase in energy creates surplus N in the ammonia and urea pool which will not be utilized by the microbes and gets excreted in urine.

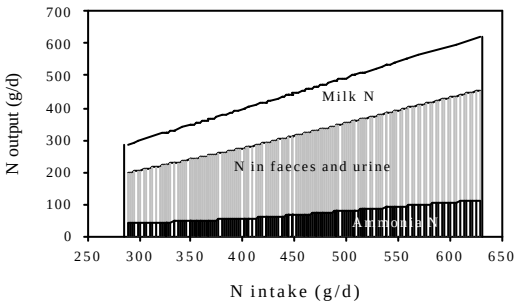


**Figure 2** Partition of N into milk, excreta and ammonia and pollution Index based on milk N produced

Changes in protein degradability marginally affected faecal and milk N output, but it had a considerable effect on urine N output such that in diets with low degradable protein contents, there was enough N in the rumen for microbial N synthesis with less N escaping the rumen and less N excreted via urine. Figure 2 indicates the relative gains of milk N and losses as ammonia N when N intake is increased. Increasing levels of N above 400-420 g/kg increases exponentially the ammonia N to Milk N ratio. When protein levels in the ration were reduced, levels of total N excretion fell from 15.2 to 12.3 g/kg milk produced, and if these results were imposed on typical livestock systems, it would represent a national net reduction in N excretion from 106 to 86 kg/animal/yr by dairy cows producing average milk yields of 7000 kg/yr. This is a reduction of nearly 15 kt ammonia/yr or 19% of the ammonia emissions arising from dairy cows.

**Conclusions** Model predictions show that availability of energy is crucial to the efficiency of of N utilization and will influence the proportion of N excreted in urine and faeces. Similarly, degradability of protein can be manipulated to affect N release in the rumen and provide additional N to be absorbed post-ruminally. This can be achieved by increasing maize in feed without adding extra protein.

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# Incremental effects of ground rapeseed on digestion in lactating dairy cows

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**Introduction** Crushed rapeseed and other oil seeds offer an economical source of fat and protein in diets for lactating dairy cows, but the potential inhibitory effects of their unsaturated fatty acids on fibre digestion in the rumen are a concern. Feeding crushed rapeseed in a grass silage-based ration increased milk yield without affecting intake (Reynolds *et al*., 1998), and had no measurable effects on rumen or total tract digestion (Reynolds *et al*., 2000). In a companion study, feeding increasing amounts of ground rapeseed in a maize silage-based ration decreased DM intake at higher levels of inclusion (Reynolds *et al*., 2002). This effect may reflect metabolic effects of rapeseed fatty acid absorption, or negative effects of rapeseed oil on rumen fermentation and fibre digestion. The present study was conducted simultaneously to the production study to determine the incremental effects of ground rapeseed on rumen, post-rumen and total tract digestion in lactating dairy cows fed maize silage-based rations.

**Materials and Methods** Three multiparous, mid-lactation Holstein-Friesian cows (616 kg body weight) with cannulas in the rumen and duodenum were used in a 3 X 4 Latin Square design study with 5 week periods. The study began with 4 cows, but one cow was dropped from the study for health reasons. Cows were fed a control diet containing 428 g maize silage, 142 g grass silage and 186 g crude protein/kg DM or the same diet supplemented with ground rapeseed to provided 25, 40 or 55 g of added oil/kg diet DM. Ground rapeseed replaced wheat and rapeseed meal in the basal diet and diets were formulated to be isonitrogenous. Digestion trials during the last week of each period included measurements of N balance and duodenal flow measured using CrEDTA and Yb acetate infusion. Feed offered during digestion trials was restricted to *ad libitum* intake during the previous week to minimize refusals. Cows were fed at 8-h intervals and milked twice daily. Data were statistically analyzed by analysis of variance to determine the probability of linear (L), quadratic (Q) and cubic (C) effects of supplemental rapeseed.

**Results** Intake of DM and N and milk and milk N yield decreased linearly with added rapeseed (Table 1). This was reflected by a decrease in total DM digestion, as well as duodenal flow of non-ammonia N. However, the respective ruminal and post-ruminal fractional digestibility (g/kg intake or g/kg duodenal flow) of DM (247 [s.e. 58] and 605 [s.e. 29]), starch (751 [s.e. 87] and 862 [s.e. 37]), NDF (238 [s.e.97] and 284 [s.e. 77]) and ADF (178 [s.e. 100] and 300 [s.e. 82]) were not affected by diet. Similarly, total digestibility (g/kg intake) of DM (705, s.e. 3), starch (972, s.e. 3), NDF (461, s.e. 17) and ADF (432, s.e. 13) and body N balance (8 g/day, s.e. 20) were not affected by diet.

**Table 1.** *Milk and milk N yield, DM and N intake, rumen and total digestion of DM and duodenal non-ammonia N flow (NAN) in lactating dairy cows fed diets containing increasing amounts of oil from ground rapeseed.*

| | g added oil/kg diet DM | | | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 25 | 40 | 55 | s.e. | L | Q | C |
| Milk yield, kg/day | 34.4 | 33.8 | 31.7 | 29.1 | 1.1 | 0.04 | 0.25 | 0.88 |
| Milk N, g/day | 178 | 172 | 159 | 151 | 4 | 0.02 | 0.40 | 0.54 |
| DM intake, kg/day | 21.9 | 20.3 | 19.3 | 18.3 | 0.4 | 0.01 | 0.98 | 0.98 |
| N intake, g/day | 640 | 578 | 552 | 499 | 10 | 0.01 | 0.54 | 0.42 |
| Rumen DM digestion, kg/day | 5.7 | 5.2 | 4.4 | 4.4 | 1.2 | 0.43 | 0.95 | 0.84 |
| Total DM digestion, kg/day | 15.6 | 14.2 | 13.6 | 12.9 | 0.3 | 0.01 | 0.72 | 0.85 |
| Duodenal NAN flow, g/day | 647 | 583 | 569 | 533 | 11 | 0.01 | 0.66 | 0.42 |

**Conclusions** In the present study feeding increasing amounts of ground rapeseed reduced DM intake and milk yield linearly. This was associated with decreases in amounts of DM digested daily, but not the digestibility of DM, starch, NDF or ADF. Although fractional digestibility is affected by level of feeding, the present data suggest that decreases in intake when ground rapeseed was fed at 40 or 55 g oil/kg DM in production studies was due to post-ruminal effects of fat absorption and metabolism, rather than inhibitory effects on NDF or ADF digestion in the rumen. In contrast to the companion production trial, DM intake and milk yield were reduced even by the lowest level of rapeseed feeding, suggesting that adaptation to rapeseed feeding may be important in determining lactation responses.

**Acknowledgements** Funded by the Milk Development Council.

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# Replacement of soyabean meal by barley or wheat distillers grains for lactating dairy cows

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**Introduction** The complete replacement of soyabean meal by maize distillers grains (MDG) in dairy cow diets had no significant effects on milk production, rumen digestion or the supply of non-ammonia N to the duodenum (Sutton *et al*., 2000; Phipps *et al*., 2001). The present experiment was designed to extend this work by examining the response when barley (BDG) or wheat (WDG) distillers grains replaced soyabean meal.

**Materials and methods** Four multiparous Holstein-Friesian cows with permanent rumen and duodenal cannulas were offered 4 diets in a 4x4 Latin square experiment with 4-week periods starting 3 months after calving. In week 4 of each period rumen and duodenal samples were taken to give 2-h intervals from 6.30 to 22.30 h spread over 3 days. Measurements of duodenal flow were based on Yb acetate and CrEDTA as markers.

The cows were offered *ad libitum* a total mixed ration (TMR) based on 350 g grass silage, 176 g maize silage and 474 g concentrates/kg (DM basis). The treatments consisted of four formulations of concentrates with soya alone (527 g crude protein (CP), 28 g oil B/kg DM), or with BDG (259 g CP, 86 g oil B/kg DM) replacing one-half or all of the soya or with WDG (320 g CP, 80 g oil B/kg DM) replacing all the soya on a N equivalent basis to give treatments Soya, Soya/BDG, BDG or MDG (Table 1). Urea and Megalac were used to balance ERDP and oil B.

**Table 1.** *Principal concentrate components of the TMR (kg/t DM) and TMR composition (g/kg DM)*

| | Soya | Soya/BDG | BDG | WDG |
|---|---|---|---|---|
| Cracked wheat | 181 | 150 | 142 | 154 |
| Molassed sugar beet feed | 100 | 94 | 63 | 79 |
| Rapeseed meal | 70 | 64 | 61 | 71 |
| Soyabean meal | 100 | 50 | 0 | 0 |
| Barley distillers grains | 0 | 96 | 192 | 0 |
| Wheat distillers grains | 0 | 0 | 0 | 155 |
| NDF | 362 | 396 | 419 | 412 |
| Crude protein | 177 | 174 | 173 | 162 |
| Oil B | 34 | 37 | 40 | 39 |

**Results** DM intake was unaffected by treatments (Table 2). Trends for higher rumen digestion of organic matter (OM) and neutral detergent fibre (NDF) with BDG and WDG than with Soya were non-significant. There were no significant effects on rumen digestion of total N or flow of non-ammonia N (NAN) to the duodenum. Rumen pH, ammonia and volatile fatty acid (VFA) concentrations and VFA molar proportions were unaffected by treatments.

**Table 2.** *Dry matter intake, digestion in the rumen and N intake and digestion*

| | Soya | Soya/BDG | BDG | WDG | s.e.d. |
|---|---|---|---|---|---|
| DM intake (kg/day) | 19.73 | 20.34 | 19.85 | 19.32 | 0.505 |
| Total N intake (kg/day) | 0.558 | 0.567 | 0.550 | 0.500 | 0.0223 |
| Rumen digestion (g/g) | | | | | |
| Organic matter | 0.389 | 0.411 | 0.430 | 0.456 | 0.0580 |
| NDF | 0.478 | 0.438 | 0.501 | 0.561 | 0.0529 |
| Total N | -0.128 | -0.116 | -0.036 | -0.163 | 0.0679 |
| NAN at duodenum (kg/day) | 0.575 | 0.584 | 0.521 | 0.538 | 0.0426 |

Milk yield and composition were unaffected by the treatments though milk yield (30.3, 29.2, 29.7, 28.7 (s.e.d. 1.09) kg/day) and fat content (40.5, 38.7, 37.3, 35.8 (s.e.d. 1.68) g/kg) tended to be lower on WDG than Soya.

**Conclusions** The complete replacement of soyabean meal by MDG, BDG or WDG has been found to have no significant effect on feed intake, rumen digestion or non-ammonia N flow to the duodenum in two experiments. There was some evidence of small, non-significant reductions in milk yield and fat content with all three forms of distillers grains but these experiments were not designed primarily to examine milk production responses. In a related feeding trial with MDG (Phipps *et al.,* 2001) no such reductions were observed.

**Acknowledgements** This work was funded by Trident Feeds.

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Sutton, J.D., Humphries, D.J. and Witt, M. 2000. Replacement of soyabean meal by maize distillers grains for lactating dairy cows. *Proceedings of the British Society of Animal Science,* p 92.

# Prediction of the intake potential of grass silage in the supplemented diets of lactating dairy cows

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**Introduction** In the majority of farming systems in Ireland and the UK, grass silage is supplemented with varying types and levels of concentrates when offered to lactating dairy cows (Keady *et al*., 1998). This supplementation results in a reduction in the intake of the silage. The aim of the current study was therefore to use data from the literature to develop empirical models that would allow the prediction of grass silage dry matter intake with increasing amounts of concentrate intake.

**Materials and Methods** The relationship between silage intake and concentrate supplementation in dairy cows was developed using treatment mean data from a study performed at the Agricultural Research Institute of Northern Ireland (Mayne *et al*., 1995) where silages (n=8) of varying quality were offered to lactating dairy cows (n=128) and different levels of concentrate intake were examined (unsupplemented, 3.5, 6.8 or 9.8 kg DM/day). A total of 19 supplement treatments were examined with each silage type, with each level of supplementation being offered at 3 protein concentrations and two energy sources. The relationship between silage intake (y) and concentrate intake (x), expressed as $gkg^{-1}$ $W^{0.75}$ was determined using regression techniques. Two different relationships between such were examined, namely, 1) the linear relationship $y=a_i+b_ix$ and 2) the exponential relationship $y=c_i+d_i\ r_i^x$, where *i* represents the silage type for *i*=1-8. Then $y=a_i$ and $y=(c_i+d_i)$ represent the silage intake when silage *i* is offered to the animal as the sole feed. The linear and exponential models were constrained to pass through a common intercept on the x-axis, representing the concentrate intake at zero silage intake, giving rise to the following equations: 1) Linear: $k_L=(-a_i/b_i)$ giving the model $y=a_i[1-(x/k_L)]$ and 2) Exponential: $k_E=\log_e(-c_i/d_i)/\log_e(r_i)$ giving the model $y=-d_i(r^{kE})-r_i^x)$, where $k_L$ (203.8g concentrate/kg $W^{0.75}$) and $k_E$ (168.0g concentrate/kg $W^{0.75}$) are the constants for the linear and exponential models respectively. An adjustment factor of 0.14kg DM/kg milk was used to adjust the silage intake potential of dairy cows for differences in milk yield (MY) and MY was standardised at 8kg/day for both the linear and exponential models. The models were validated with an independent data set (Patterson *et al*., 1998), where 32 grass silages were offered to lactating dairy cows (n=48) and silage intakes predicted from each developed model were compared with actual measured intakes.

**Results** There was no significant effect of protein concentration or energy source on silage intake ($P > 0.05$). The fitted linear and exponential models had $R^2$ values of 0.750 and 0.756, when all points were constrained through a common intercept on the x-axis ($k_L$ and $k_E$) as shown in Figures 1 and 2. Validation of the applied models showed that the actual and predicted intakes were not significantly different from zero ($P > 0.05$).

**Figure 1** Linear Model

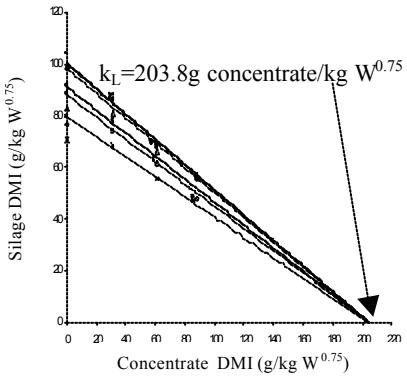
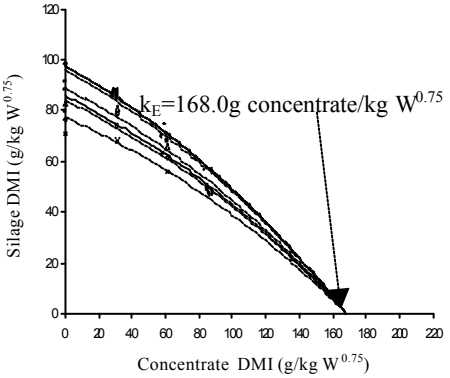



**Table 1** Goodness of fit of models

| Model | Predicted Mean (Actual) |
|---|---|
| Linear | 92.6 (98.8) |
| Exponential | 95.0 (98.8) |
| **Model** | **Predicted s.e. (Actual)** |
| Linear | 1.89 (2.26) |
| Exponential | 1.95 (2.26) |

**Figure 2** Exponential Model




**Conclusions** Although the linear and exponential models had similar abilities to predict silage dry matter intake, the exponential model was moderately more accurate (Table 1) and would also seem more appropriate from a biological perspective. Model validation showed that 93% of the intake predictions were within 10% of actual intakes.

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## Production response of lactating dairy cows fed increasing amounts of ground rapeseed.

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**Introduction** Fat is often fed to lactating dairy cows to provide supplemental metabolizable energy and increase milk yield. Commercial fat sources are typically processed to render them more inert in the rumen, but whole oil seeds also represent an effective fat source for lactation rations. In a previous study (Reynolds *et al*., 1998), we fed lactating dairy cows a commercial rumen-inert fat, cotton seed or crushed rapeseed at 25 g added fat/kg ration DM in a grass silage-based total mixed ration (TMR). All 3 fat supplements increased milk yield to a similar extent, whilst the commercial fat source, but not the whole oil seeds, reduced milk protein concentration. As a UK grown source of supplemental fat and protein, crushed rapeseed offers an attractive alternative to commercial fat supplements for dairy rations. Therefore, our objective was to determine the incremental effects of supplemental ground rapeseed on intake and milk production response of dairy cows fed rations containing maize silage.

**Materials and Methods** Forty multiparous Holstein-Friesian cows were used in a randomized block design study with 10 cows per treatment. From calving to week 5 postpartum cows were fed a control maize silage-based TMR (428 g maize silage, 142 g grass silage and 186 g crude protein/kg DM) for *ad libitum* intake. During week 6 postpartum cows were incrementally changed to one of 4 diets in blocks based on parity, body weight and milk yield. Treatments were the control diet or the control diet supplemented with 25, 40 or 55 g rapeseed oil/kg diet DM by substituting a ground rapeseed and wheat blend for ground wheat and rapeseed meal in the control diet. Rations were fed once daily for *ad libitum* intake and milk yield measured twice daily through week 20 postpartum. Milk composition, body weight and condition score were measured weekly. Data were statistically analyzed as repeated measures to determine linear, quadratic or cubic effects of supplemental rapeseed oil using Mixed Models procedures and average measurements for weeks 4 to 5 postpartum as a covariate.

**Results** Daily DM intake was reduced by feeding 40 or 55 g of added oil/kg diet DM, but milk yield was not affected by feeding rapeseed (Table 1). Milk fat concentration was decreased by feeding ground rapeseed and the effect tended to be quadratic, with a large reduction (5.2 g/kg) when 25 g of added oil/kg diet DM was fed and a further reduction of 1.5 g/kg at the highest level of inclusion. In contrast milk fat yield was reduced linearly by the addition of increasing amounts of rapeseed. Milk protein concentration and yield were not affected by diet.

**Table 1.** Intake and milk production in dairy cows fed diets containing added oil from ground rapeseed during weeks 7 to 20 of lactation.

| | g added oil/kg diet DM | | | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 25 | 40 | 55 | s.e. | Linear | Quadratic | Cubic |
| DM intake, kg/d | 17.2 | 17.1 | 15.5 | 15.4 | 0.3 | 0.001 | 0.844 | 0.051 |
| Milk yield, kg/d | 28.9 | 30.6 | 28.5 | 28.2 | 1.0 | 0.310 | 0.340 | 0.196 |
| Milk composition, g/kg | | | | | | | | |
| Fat | 38.0 | 32.8 | 32.0 | 31.3 | 1.2 | 0.001 | 0.062 | 0.377 |
| Protein | 33.3 | 32.6 | 32.6 | 32.9 | 0.6 | 0.609 | 0.422 | 0.831 |
| Milk component yield, g/d | | | | | | | | |
| Fat | 1085 | 989 | 914 | 884 | 39 | 0.001 | 0.395 | 0.883 |
| Protein | 965 | 990 | 922 | 918 | 26 | 0.081 | 0.589 | 0.167 |

**Conclusions** When fed in a grass silage-based TMR crushed rapeseed fed to provide 25 g added oil/kg diet DM increased milk yield by 1.5 kg/d and did not affect milk fat concentration (Reynolds *et al*., 1998). In the present study the same level of inclusion in a maize silage-based ration numerically increased milk yield by a similar amount, but the overall effect of feeding increasing amounts of rapeseed on milk yield was not significant. In contrast, milk fat concentration was reduced when ground rapeseed was fed in the present study. This may reflect a higher oil (28 vs 39 g/kg DM) and starch (176 vs 285 g/kg DM) content of the basal diet fed in the present study, and subsequent effects on rumen fermentation, or differences in oil availability between crushed and ground rapeseed. Intake was reduced by higher levels of rapeseed inclusion, which may reflect digestive or metabolic effects of the supplemental oil. These observations suggest that crushed or ground rapeseed can be included in grass or maize silage-based rations for lactating dairy cow diets at 25 g of added oil/kg diet DM without affecting intake, but milk fat concentration may be reduced when rapeseed is added to maize- based diets with a high starch content. Higher levels of rapeseed inclusion in maize silage-based rations reduced intake and were of no benefit to milk yield.

**Acknowledgements** Funded by the Milk Development Council.

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Reynolds, C. K., Phipps, R. H., Jones, A. K. and Beever, D. E. 1998. Milk production response of lactating dairy cows to dietary fat from 3 sources. *Proceedings of the British Society of Animal Science*, p. 223.

# Response of dairy cows offered a high feed value grass silage, to concentrate feed level and concentrate crude protein content

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**Introduction** The environmental and economic cost of concentrate protein ingredients is high, with the economic impact being especially important if these are certified free from genetically modified material. While reducing the crude protein (CP) content of the concentrate will reduce feed costs, animal performance is also likely to suffer (Mayne and Gordon, 1985). However it may be possible to maintain performance and yet reduce feed costs by reducing the protein content of the concentrate offered, with a simultaneous increase in concentrate feed levels. The current study was designed to examine the effects on animal performance of adopting this approach, and to quantify the 'protein sparing' effect of increasing the quantity of concentrate offered.

**Material and methods** Five treatments were examined in a 3 period (period length, 4 weeks) partially balanced change-over design trial involving 15 Holstein-Friesian dairy cows of mixed parity [mean of 87 (s.d., 29.7) days calved at the start of the experiment]. With treatments 150(6), 200(6) and 250(6), 6.0 kg/cow/day of a concentrate containing 150, 200 and 250 g CP/kg fresh respectively was offered (calculated degradability of protein, 0.60, 0.58 and 0.56 respectively). With treatments 150(8) and 150(10), 8.0 or 10.0 kg/day respectively of a concentrate containing 150 g CP/kg was offered (fresh basis). The ingredient composition (kg/t air dry basis) of the high protein (250 g CP/kg) concentrate was: wheat, 310; maize gluten, 140; maize distillers, 70; soya bean meal, 390; minerals, 30; Megalac, 40; molasses, 40. The low protein concentrate (150 g CP/kg) was similar except that the wheat and soya bean meal inclusion rates were 590 and 110 kg/t respectively. The medium protein concentrate (200 g CP/kg) was produced by mixing the other two concentrates in equal proportions. These concentrates were offered through an out-of-parlour feed system, while a high feed value grass silage (dry matter, CP and ammonia nitrogen concentrations of 413 g/kg, 143 g/kg DM and 56 g/kg total nitrogen) was offered *ad libitum* via a Calan gate feed system. Data from this study was analysed as a five-treatment change-over design experiment using Analysis of Variance.

**Results** Silage DM intake was unaffected by concentrate CP content but decreased with increasing concentrate feed level ($P<0.001$). Both milk yield and milk energy output showed a significant increase with both concentrate feed level and CP content ($P<0.001$), while neither milk fat, milk protein nor milk energy concentrations were affected by treatment. Using the treatment mean data, the milk energy output response to increasing levels of the concentrate containing 150 g CP/kg can be described by the quadratic equation: $Y = -0.325x^2 + 0.7.1x + 53.1$, where Y = milk energy output (MJ/cow/day) and x = intake of the concentrate (kg fresh/day) containing 150 g CP/kg. By interpolation, an equivalent milk E output as was achieved from offering 6.0 kg/day of a concentrate containing 200 and 250 g CP/kg (namely 88.6 and 91.4 MJ/cow/day respectively), could have been achieved through offering 7.7 or 9.7 kg/day (fresh basis) respectively of a concentrate containing 150 g CP/kg.

**Table 1** Treatment effects on feed intake and milk output

| | Treatment | | | | | | |
|---|---|---|---|---|---|---|---|
| | 150(6) | 200(6) | 250(6) | 150(8) | 150(10) | S.E.M | SIG. |
| Intake (kg DM/day) | | | | | | | |
| Concentrate | 5.21 | 5.20 | 5.19 | 6.90 | 8.60 | | |
| Silage | $12.3^{bc}$ | $12.3^{bc}$ | $12.9^{c}$ | $11.5^{b}$ | $10.4^{a}$ | 0.23 | *** |
| Total | $17.5^{a}$ | $17.4^{a}$ | $18.1^{ab}$ | $18.4^{b}$ | $19.0^{b}$ | 0.30 | ** |
| Milk yield (kg/day) | $26.4^{a}$ | $27.6^{b}$ | $28.2^{b}$ | $28.3^{b}$ | $29.4^{c}$ | 0.35 | *** |
| Milk composition | | | | | | | |
| Butter fat (g/kg) | 40.9 | 41.4 | 42.7 | 39.2 | 38.5 | 1.11 | NS |
| Protein (g/kg) | 34.1 | 33.8 | 33.9 | 33.8 | 34.0 | 0.24 | NS |
| Energy (MJ/kg) | 3.21 | 3.22 | 3.27 | 3.15 | 3.13 | 0.042 | NS |
| Milk E output (MJ/day) | $84.0^{a}$ | $88.6^{b}$ | $91.4^{b}$ | $89.1^{b}$ | $91.6^{b}$ | 1.45 | *** |

**Conclusions** Similar levels of milk energy output as achieved from offering either 6.0 kg/day of a concentrate containing 200 or 250 g CP/kg could have been achieved by reducing the concentrate CP content to 150 g/kg, and by increasing the concentrate feed level to 7.7 and 9.7 kg/day respectively. This indicates that there is an opportunity to reduce total nitrogen inputs from concentrate feed stuffs, and, depending on the relative costs of the different feedstuffs and concentrate ingredients, reduce costs.

**Reference** Mayne, C.S. and Gordon, F.J. 1985. The effect of concentrate-to-forage ratio on the milk-yield response to supplementary protein. Animal Production **41**: 269 - 279.

**Acknowledgements** This work was co-funded by AgriSearch, MDC, DARD and IFI Ltd

# Response of grazing dairy cows to level of concentrate supplementation and concentrate protein content

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**Introduction** The response of grazing dairy cows to level of concentrate supplementation and to the concentration of protein in the supplement remains poorly defined. The response is of particular importance in view of the high costs associated with the concentrate feedstuffs, especially protein ingredients. The current study was designed to examine the response of grazing dairy cows to level of concentrate supplementation and to level of protein in the concentrate.

**Material and methods** Eighteen Holstein-Friesian dairy cows (mean $PTA_{2000}$ fat + protein, 28.0 kg; mean interval from calving, 240 days) were used in a 3 period (period length = 4 weeks) partially balanced change-over design trial. Six of the animals were in their first lactation, while the remaining 12 had a mean lactation number of 2.2. Six treatments were examined in this 2 x 3 factorial design experiment: two levels of concentrate supplementation [3.6 (Low) and 7.2 (High) kg fresh concentrate/day] and three levels of crude protein (CP) in the concentrate (110 , 170 and 230 g/kg fresh weight). The ingredient composition (kg/t air dry basis) of the low CP concentrate (110 g/kg) was: barley, 150; maize, 255; sugar-beet pulp, 430; soya bean meal, 95; minerals, 25; calcined magnesite, 10; molasses, 35. The equivalent values for the high CP concentrate (230 g/kg) were 90, 165, 270, 405, 25, 10, and 35 kg/t respectively. The medium CP concentrate (170 g/kg) was produced by mixing the high and low protein concentrates in equal proportions. The concentrate supplements were offered in-parlour during the am and pm milking, as two equal feeds. The animals were rotationally grazed on perennial ryegrass swards with the aim of achieving a residual sward height, measured using a plate meter, of between 5 - 7 cm.

**Results** Pre- and post-grazing sward heights, measured daily throughout the study, were 15.6 and 6.1 cm respectively, while the mean herbage allocation per animal, measured above a height of 4.0 cm, was 17.0 kg DM/cow/day. There were no significant interactions between concentrate level and concentrate CP content for any of the parameters measured. Milk yield showed a significant increase (both linear $P<0.01$ and quadratic $P<0.05$ trends) with increasing concentrate CP level, and with increasing concentrate level. The latter response was equivalent to 0.72 kg milk/kg concentrate DM, while the response to concentrate CP content in excess of 170 g/kg was small or negative. Milk fat concentration exhibited a slight quadratic trend ($P<0.05$) with increasing concentrate protein level, decreasing with increasing concentrate level ($P<0.05$). Neither milk protein nor milk energy concentrations were affected by treatment ($P<0.05$). Each of milk fat, protein and energy yields exhibited significant linear increases with increasing concentrate CP content ($P<0.01$), while increasing with concentrate feed level ($P\leq0.05$).

**Table 1** Treatment effects on animal performance

| | Low concentrate level (3.6 kg/day) | | | High concentrate level (7.2 kg/day) | | | | Protein content | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 110 | 170 | 230 | 110 | 170 | 230 | SED | Linear | Quadratic | Conc. level |
| Milk yield (kg/day) | 19.7 | 21.9 | 20.8 | 21.2 | 23.8 | 24.1 | 0.91 | ** | * | *** |
| Milk composition | | | | | | | | | | |
| Fat (g/kg) | 40.7 | 39.3 | 40.9 | 39.0 | 37.1 | 39.6 | 1.45 | NS | * | * |
| Protein (g/kg) | 35.1 | 36.0 | 35.3 | 35.2 | 35.3 | 35.0 | 0.66 | NS | NS | NS |
| Energy (MJ/kg) | 3.21 | 3.17 | 3.21 | 3.15 | 3.08 | 3.17 | 0.064 | NS | NS | NS |
| Milk constituent yield | | | | | | | | | | |
| Fat (g/day) | 790 | 846 | 850 | 826 | 876 | 941 | 43.0 | ** | NS | * |
| Protein (g/day) | 693 | 775 | 722 | 742 | 834 | 841 | 28.8 | ** | ** | *** |
| Energy (MJ/day) | 62.7 | 68.6 | 66.5 | 66.8 | 72.8 | 75.7 | 2.78 | ** | NS | *** |

**Conclusions** The milk yield response in the current study (0.72 kg milk/kg concentrate DM) was considerably greater than the mean response reported previously (Leaver *et al*., 1968) for low yielding cows. This may reflect the fact that animals were grazing a relatively low herbage allowance (17.0 kg DM/cow/day), or that high genetic merit animals may be able to continue to exhibit considerably greater responses to supplementation, even in late lactation. In addition, the data suggest that there is little benefit in increasing the CP content of grazing concentrates to more than 170 g/kg.

**Acknowledgements** This work was co-funded by AgriSearch, MDC, DARD and IFI Ltd

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# The reduction of nutrient digestibility and energy concentration from maintenance feeding (sheep) to production feeding (lactating dairy cows) in grass silage-based diets

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**Introduction**. AFRC (1993) recommends a reduction of proportionately 0.018 in dietary metabolisble energy (ME) concentration with each unit increase in feeding level above maintenance in dairy cows (feeding level is calculated as total ME intake divided by ME requirement for maintenance). A similar value (0.016) was reported recently by Yan *et al*. (2001) using a number of linear and multiple regression techniques with lactating dairy cows offered grass silage-based diets. The objectives of the present study were to validate these two values and also to evaluate the effects of feeding level on nutrient digestibility and ME concentration in the mixed diets.

**Material and methods** A total of 12 grass silages, each supplemented with concentrates, were offered *ad libitum* to lactating dairy cows (3 animals/silage) in two separate experiments, with the aim of measuring the effects of silage quality and concentrates on nutrient digestibility and urinary energy output. In addition, the 12 silages were also offered individually to sheep as the sole feed at maintenance feeding level, and nutrient digestibility and urinary energy output measured. The nutrient digestibility and ME concentration of mixed diets were estimated using the measured silage data through sheep and tabulated energy values of concentrates. The methane energy outputs for the silages (sheep) and mixed diets (cattle) were predicted. Treatment mean data were used in the present study (n = 13, one silage was offered with two types of concentrates, while the other 11 silages each with one type of concentrates).

**Results** The results are presented in Table 1. With the mixed diets across the two trials, digestibility of dry matter (DM), organic matter (OM), nitrogen (N) and neutral detergent fibre (NDF) and digestible OM in total DM (DOMD) and concentration of ME and digestible energy (DE) determined in cows at production (Prod) level were all significantly lower than those estimated in sheep at maintenance (Maint) ($P<0.01$ or less). The extents of these reductions ((Maint-Prod)/Maint), with a mean feeding level of 3.4 (AFRC, 1993), ranged from 0.035 (DM digestibility) to 0.063 (N digestibility). Similarly, with each unit increase in feeding level above maintenance, dietary ME and DE concentrations and DOMD were reduced by proportionately 0.015, 0.020 and 0.017 respectively, when assuming that there was no significant difference in any nutrient digestibility between sheep and cattle fed at maintenance (e.g., energy digestibility, Yan *et al*., 2001). The corresponding values for digestibility of DM, OM, N, and NDF were respectively 0.015, 0.017, 0.027 and 0.022. These ME and DE data were also used to validate the feeding level correction factors of AFRC (1993) (ME 0.018) and Yan *et al.* (2001) (ME 0.016 and DE 0.025) using the mean-square-prediction-error (MSPE). The validation indicated a small line (slope) error for all three factors, but a relatively large error derived from bias (actual – predicted) over total MSPE for the ME 0.018 (AFRC, 1993) (0.09) and DE 0.025 (Yan *et al.,* 2001) (0.23). The ME 0.016 (Yan *et al.,* 2001) predicted well with a random error over total MSPE of 0.98.

**Table 1**. Effects of feeding level (FL) on various variables obtained at production (cattle) and maintenance (sheep) FL

| | Feeding level | | | | (Maint-Prod)/Maint | |
|---|---|---|---|---|---|---|
| | Prod. | Maint. | s.e.d | Sig. | All FL | One FL[#] |
| Digestibility (kg/kg) | | | | | | |
| Dry matter | 0.764 | 0.792 | 0.0066 | *** | 0.035 | 0.015 |
| Organic matter | 0.792 | 0.825 | 0.0063 | *** | 0.040 | 0.017 |
| DOMD | 0.724 | 0.755 | 0.0054 | *** | 0.041 | 0.017 |
| Nitrogen | 0.732 | 0.781 | 0.0092 | *** | 0.063 | 0.027 |
| Gross energy | 0.763 | 0.801 | 0.0065 | *** | 0.048 | 0.020 |
| Neutral detergent fibre | 0.706 | 0.745 | 0.0112 | ** | 0.052 | 0.022 |
| Concentration (MJ/kg DM) | | | | | | |
| Metabolisable energy | 11.93 | 12.37 | 0.156 | ** | 0.036 | 0.015 |
| Digestible energy | 14.04 | 14.74 | 0.165 | *** | 0.048 | 0.020 |

[#] One unit of feeding level above maintenance

**Conclusion** With each unit increase in feeding level above maintenance, nutrient digestibility can be reduced from 0.015 (DM) to 0.027 (N) and ME concentration by 0.015 in dairy cows offered grass silage-based diets.

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# Comparison of Calan gate and easy feed systems on the intake of dairy cows

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**Introduction** In many Research Centres in the UK, dairy and beef cattle access their daily allowance of forage, and perhaps concentrates, through Calan gate type feeding systems. As up to four animals may share one feed unit in these systems, only a proportion of animals can gain access to feed at any one time. This may have a negative effect on food intake and subsequent animal performance. The present study was undertaken to evaluate the effects on intake from offering food through a Calan gate type system, compared to a conventional easy feed system.

**Material and methods** Twenty-four late lactation dairy cows [285 days calved (s.d., 26.8); mean milk yield, 18.6 (s.d., 2.98) kg] were used in a two treatment (12 animals per treatment), three period (period length, 14 days), change over design experiment. The two treatment groups were housed separately, side by side, in cubicle accommodation, and offered a total mixed ration (TMR) consisting of grass silage and concentrates (60 : 40 on a dry matter (DM) basis). Animals had access to feed for 22 h/day, between 11.00 - 09.00 h. Fresh feed was offered *ad libitum* daily, at 11.00 h. The cows were milked between 17.00 - 18.00 h, and between 06.00 - 07.00 h, and did not have access to feed during this period. The treatments examined (CG and EF) involved two different methods of allowing animals access to their diets. With treatment CG, the 12 animals in the group accessed their feed via three Calan gates (width of access space/gate, 22.8 cm), a maximum of three animals being able to feed at any one time. Through each Calan gate, animals accessed a feed box specific to that gate (feed box dimensions: length, 120 cm; depth, 104 cm; width at top, 118 cm; width at base, 63 cm) containing the TMR. Each feed box was mounted on a weigh platform, which was linked to an automatic cow identification system, thus allowing individual animal intakes to be recorded. With treatment EF, three Calan gates and their surrounding fittings were removed, leaving a feed space 366 cm long (30.5 cm/cow). Animals accessed their feed through this feed space, with a maximum of 8 animals being able to feed at any time. The TMR was placed in a single feed box (feed box dimensions: length, 385 cm; depth, 60 cm; width at top, 94 cm; width at base, 63 cm) which rested on two weigh platforms. This allowed the weight of feed in the box to be measured, but did not permit the recording of individual animal intakes. Feed intakes were recorded during the second week (recording week) of each period. In addition, during day 5 of the recording week, the weight of feed consumed hourly by each group of animals was recorded during the 22 hour period (11.00 - 09.00) when animals had access to feed. During this same period the number of animals feeding within each group was recorded at 10 minute intervals using the 'group scan' technique.

**Results** Feeding systems did not affect food intake, with mean intakes of 15.6 (s.e., 0.12) and 15.4 (s.e., 0.34) kg DM/day for treatments CG and EF respectively ($P>0.05$). Intakes of animals on treatment CG were lower ($P<0.001$) between 11.00 and 12.00 h (Figure 1), but were higher between 04.00 and 05.00 h ($P<0.05$), relative to intakes with treatment EF. Intakes were not different during any other hourly period ($P>0.05$). Between 11.00 - 20.00 h the mean number of animals observed feeding each hour (Figure 2) was significantly higher with treatment EF compared with treatment CG ($P<0.001$), while the reverse was true between 04.00 and 05.00 h ($P<0.001$).

**Figure 1** Mean hourly intakes over a 22 hour period (EF, ¦ ; CG, ? )

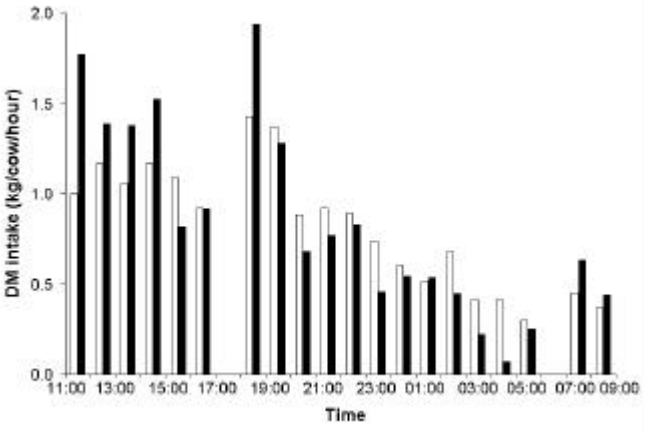
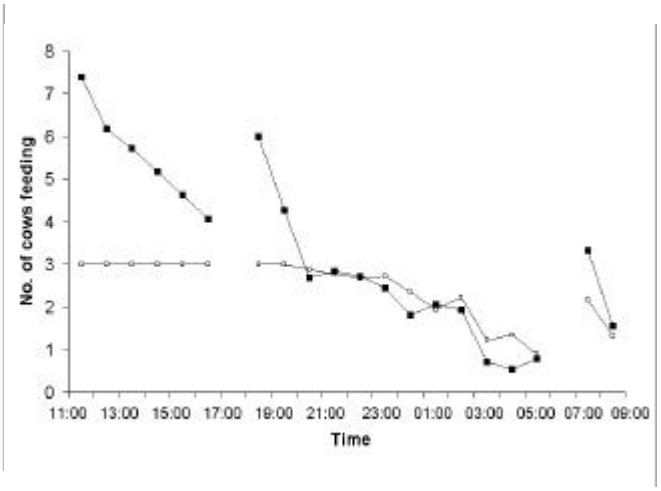



**Figure 2** Mean number of cows observed feeding each hour over a 22 hour period (EF, ¦ ; CG, ?)




**Conclusions** Despite large differences in available feed places between the two systems (EF, 8; CD, 3), total DM intake was unaffected by feeding system. While the use of a Calan gate type feeding system did not inhibit intake compared to a conventional 'easy feed' type system, the feeding system used did influence the cows feeding behaviour.

**Acknowledgements** This work was co-funded by AgriSearch, MDC, DARD and IFI Ltd.

# The potential of a range of maize silages, included as a component of grass silage based diets, to increase the dry matter and energy intakes of finishing beef cattle

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**Introduction** The inclusion of maize silage in grass silage based diets may offer the potential to improve productivity on farms due to associative effects resulting from mixed-forage diets on nutrient supply to the animal. The objective of the present study was to examine the effects on dry matter (DM) and metabolisable energy (ME) intakes of beef cattle of offering forage mixtures comprising grass and maize silages of differing qualities.

**Materials and methods** 72 continental cross beef cattle, mean initial live weight 467 (sd 30.8) kg, were blocked according to live weight and used in a 2-period (4 weeks/period), changeover design experiment. The 16 treatments were arranged as a 4 x 4 factorial design incorporating 3 maize (M) silages and zero M silage, times four grass (G) silages, offered in a 60:40 ratio (DM basis) of G:M once daily through individual Calan gates. The forage component of the diet was offered *ad libitum*, and all diets were supplemented with 3kg/head/d concentrates. Concentrate composition was varied between diets such that the crude protein (CP) content of the diet with the lowest CP content was as close as possible to 140g/kg DM. The mean chemical composition of the concentrate offered throughout the study was: DM 868.8g/kg, CP 208.4, ADF 83.3, NDF 182.3, starch 282.1 and ash 70.1g/kg DM. The mean DM (g/kg), $NH_3$-N (proportion of total N), pH and lactic acid contents of the 4 grass silages were (G1) 157.2, 0.17, 4.68 and 10.7; (G2) 176.5, 0.17, 4.52 and 26.5; (G3) 244.7, 0.16, 4.29 and 37.4; and (G4) 243.5, 0.13, 4.23 and 71.0 respectively. The DM (g/kg) and starch (g/kg DM) contents of the 3 maize silages were 237.4 and 137.5, 238.7 and 195.4, and 388.3 and 337.3, for M1, M2 and M3 respectively. Total diet ME content and DM digestibility (DMD) were determined through 16 different cattle in a 3-period changeover design experiment incorporating 6-d balance studies. Feed intake results obtained in the final week of each period were used in the statistical analysis of the data. Data were analysed using the REML technique in Genstat 5 (release 4.1, Rothamsted, England) with forage type (G or M) as the main factor.

**Results** Data on total diets offered are presented in Table 1. In general, ME content was not influenced by type of M silage offered in conjunction with each individual G silage. However, ME contents of diets based on G1 were significantly lower (mean 11.38 MJ/kg DM) than diets based on any of the other individual G silages (at least $P<0.05$). Diets containing G1, G2 and G4 as the sole forage had the highest CP contents ($P<0.001$), while diets containing forage mixtures based on G3 had the lowest values. Diets based on G1 had lower DMD than all other diets offered (at least $P<0.05$), while diets containing M3 had significantly higher DMD's than diets containing either M2 ($P<0.05$) or M1 ($P<0.001$). The highest total DM and ME intakes were recorded with diets based on G4 (9.02 kg/d and 105.8 MJ/d respectively) compared with diets containing G1, G2 or G3 (at least $P<0.05$). In contrast, the mean intakes achieved between diets based on G silage offered as the sole forage, and those based on M1 and M2 were similar ($P>0.05$), but lower than that of diets containing M3 (at least $P<0.05$). Total DM and ME intakes were numerically, and in most instances significantly, higher when G4 was offered as the sole forage. However, total intakes tended to be improved with inclusion of M silage in diets based on G1, G2 and G3, and there were highly significant ($P<0.001$) interactions between some individual G-M forage mixtures resulting in increased intakes compared with G silage as the sole forage.

**Table 1** Data on total diets offered in the present study

| G | M | [ME] (MJ/kg DM) | [CP] (g/kg DM) | DMD (g/kg) | Total DMI (kg/d) | Total MEI (MJ/d) |
|---|---|---|---|---|---|---|
| 1 | - | 11.44 | 179.0 | 0.700 | 6.58 | 75.1 |
| | 1 | 11.28 | 154.0 | 0.690 | 7.24 | 81.5 |
| | 2 | 11.19 | 155.3 | 0.690 | 6.95 | 78.0 |
| | 3 | 11.60 | 151.3 | 0.709 | 7.68 | 89.1 |
| 2 | - | 11.70 | 175.9 | 0.745 | 7.82 | 91.6 |
| | 1 | 11.55 | 155.6 | 0.727 | 8.28 | 95.7 |
| | 2 | 11.70 | 152.5 | 0.742 | 7.86 | 92.0 |
| | 3 | 11.70 | 149.8 | 0.739 | 7.89 | 92.3 |
| 3 | - | 11.81 | 156.6 | 0.732 | 8.29 | 97.9 |
| | 1 | 11.95 | 138.2 | 0.733 | 8.59 | 102.5 |
| | 2 | 11.71 | 136.6 | 0.735 | 8.27 | 96.8 |
| | 3 | 11.98 | 135.6 | 0.745 | 9.16 | 109.8 |
| 4 | - | 11.98 | 183.1 | 0.752 | 9.51 | 114.0 |
| | 1 | 11.41 | 154.9 | 0.735 | 8.40 | 95.8 |
| | 2 | 11.86 | 152.9 | 0.745 | 8.93 | 105.9 |
| | 3 | 11.66 | 153.4 | 0.751 | 9.23 | 107.6 |
| sed | | 0.208 | 5.59 | 0.0073 | 0.312 | 3.63 |
| Sig. | G | *** | *** | *** | *** | *** |
| | M | NS | *** | *** | ** | *** |
| | GxM | ** | *** | *** | *** | *** |

**Conclusions** The results of the present study show some evidence of higher intakes in beef cattle when offered forage mixtures based on G and M silages of particular qualities (compared with G silage as the sole forage). There is no obvious explanation for these observations. High quality G silage produced the highest intake of all diet types offered.

# Processed urea treated whole-crop wheat (Alkalage) for finishing beef cattle

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**Introduction** Since feed accounts for 75-85% of the variable costs of beef production, the use of alternative feeds and high-energy forages that have a lower cost per unit of energy are worthy of investigation. The technique of harvesting cereals where the whole-crop is harvested at a dry matter (DM) content of 650-750g/kg with the harvester fitted with a grain processor has recently been developed. The objective of this trial was to determine the effect of feeding either *ad-libitum* processed urea treated whole-crop wheat (Alkalage) or cereals on the performance of Continental cross beef cattle since there is no data on the performance of beef cattle fed Alkalage.

**Materials and Methods** Twenty Bazadaise cross Hereford/Friesian steers born from March–April 2000 with a mean live weight of 332kg were allocated by liveweight to the following dietary treatments in a randomised block design. Cereals: *Ad-libitum* concentrates: 850g/kg rolled barley, 80g/kg soya-bean meal, 50g/kg molasses, 20g/kg minerals (DM 857 g/kg; 13.0 MJ ME/kg DM; 146 g CP/kg DM; 458 g starch/kg DM). Alkalage: *Ad-libitum* Alkalage (DM 709 g/kg; 11.1 MJ ME/kg DM; 134 g CP/kg DM; 420 g starch/kg DM; 342 g NDF/kg DM) plus 500g Lactofeed 70 (a blend of 4 parts Whey Permeate and 1 part Hi-Pro soya-bean meal which supplies 350g lactose in a feed rate of 500g per head per day, Volac International Ltd.) plus 150g High Calcium beef mineral (including Sulphur) per head per day. The treatment rations were fed to the cattle for 110 days and both groups taken through to slaughter on ad-libitum cereals. The cattle were housed in straw-bedded pens with access to water and barley straw from racks. The Alkalage was made from the winter wheat variety Equinox, cut to leave a stubble height of 37cm and ensiled with 35kg/t Home 'N' Dry (a mixture of urea and urease, Volac International Ltd.). The crop was combined with a harvester fitted with a grain processor. The data was analysed using analysis of variance

**Results**. The cattle recorded similar daily live weight gains (DLWG), with no significant differences between the treatment groups. The conformation and fat scores of the carcases equated to an R4L/4H grade on the EUROP carcase classification scheme.

**Table 1** Feed Intakes (kg/head)

| | Alkalage | Cereals |
|---|---|---|
| Total feed intake: Alkalage | 987 | |
| Lactofeed | 55 | |
| Minerals | 16.5 | |
| Cereals | 495 | 1336 |
| Straw | 65 | 175 |
| Total feed intake (DM) | 1248 | 1295 |
| Mean daily feed intake (DM) | 7.11 | 7.40 |
| Calculated FCR (kg DM feed/kg gain) | 6.31 | 6.38 |

**Table 2** Animal performance

| | Alkalage | Cereals | s.e.d | Sig. |
|---|---|---|---|---|
| Start weight (kg) | 332.7 | 332.0 | 2.94 | NS |
| Slaughter weight (kg) | 530.1 | 534.7 | 9.53 | NS |
| Days to slaughter | 175.40 | 175.00 | 1.327 | NS |
| DLWG (kg) (110 days) | 0.967 | 1.134 | 0.0980 | NS |
| Overall DLWG (kg) | 1.126 | 1.159 | 0.0513 | NS |
| Carcase weight (kg) | 288.1 | 294.0 | 7.04 | NS |
| Killing out % | 54.35 | 54.99 | 0.523 | NS |
| Conformation* | 5.00 | 5.10 | 0.233 | NS |
| Fat classification* | 4.50 | 4.50 | 0.298 | NS |

* EUROP carcase classification: Conformation: -P=1, P+=2, -O=3. O+=4, R=5, -U=6, U+=7, E=8; Fat class: 1=1, 2=2, 3=3, 4L=4, 4H=5, 5L=6, and 5H=7.

Feed costs per kg live weight gain were calculated at 54.3 and 66.0p/kg for Alkalage and Cereals respectively based on the feed prices prevailing at the time of the trial with Alkalage @ £55/t DM and cereal mix @ £95/t.

**Conclusions** Alkalage would appear to offer beef producers the opportunity to achieve high levels of animal performance and reduce feed costs per kg live weight gain.

**Acknowledgments** Financial support from Volac International Ltd. is gratefully acknowledged.

# Effect of a yeast culture on the performance of early-weaned beef calves

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**Introduction** Artificial rearing is a common practice for rearing calves from the dairy herd destined for beef production or as replacements for the dairy or suckler herds. One of the major expenses with calf rearing is the cost of the milk. Hence emphasis is placed on early weaning of the calf at 5-7 weeks old and encouraging concentrate intake. With increasing consumer concern over the use of antibiotics in feed, there is greater focus on the use of probiotics or yeast cultures to enhance calf performance. Yeast culture is a fermentation product resulting from the inoculation of grains with *Saccharomyces cerevisiae* and its growth media. This yeast culture mash is incubated and dried in a manner that preserves all the metabolites and the fermenting activity of the yeast. The objective of this study was to determine the effect of feeding a yeast culture (YC) on the performance of early-weaned beef calves.

**Materials and Methods** Twenty eight Limousin cross Holstein bull and heifer calves were randomly assigned in a 2 x 2 factorial designed experiment to either a control ration or control plus yeast culture (Diamond V XP Yeast Culture) treatment with 10 g per head per day of yeast culture. The calves started the trial at 1 day of age and were individually penned on straw. From days 1 to 4, 5 to 7 and 8 to 35 they received 2, 3 and 4 litres respectively of whole milk (DM 14.1g/kg, 42.0g butterfat/kg, 35.2g protein/kg, lactose 43.6g/kg) split into two daily feeds. From day 2 the calves received *ad libitum* coarse mix concentrates (DM 898 g/kg; 12.7 MJ ME/kg DM; 182 g crude protein/kg DM; 28 g ether extract/kg DM; 90.4 g crude fibre/kg DM) plus water. The concentrates were fed *ad libitum* to the cattle through to 12 weeks old and the yeast culture was mixed daily into the concentrate. The data was analysed by ANOVA.

**Results**. The calves receiving yeast culture had significantly higher daily liveweight gains (DLWG) from birth to 5 weeks and birth to 12 weeks old, with the calves being heavier at 5 weeks and 12 weeks. There was an increase in feed intake with yeast culture but this was not significant. The bull calves recorded significantly higher birth, 5 week and 12 week weights compared to the heifers.

**Table 1** Liveweight (kg) and daily liveweight gain (DLWG, kg)

| | YC | | Control | | | Significance | | |
|---|---|---|---|---|---|---|---|---|
| | Male | Female | Male | Female | s.e.d | Sex | Yeast | SxY |
| Birth weight | 48.47 | 44.57 | 47.49 | 43.31 | 0.681 | *** | NS | NS |
| 5 week weight | 62.44 | 59.30 | 57.49 | 51.67 | 2.004 | *** | *** | NS |
| 12 week weight | 103.80 | 99.50 | 92.30 | 84.60 | 5.750 | * | ** | NS |
| DLWG 0-5 weeks | 0.399 | 0.421 | 0.278 | 0.267 | 0.0601 | NS | ** | NS |
| DLWG 5-12 weeks | 0.843 | 0.818 | 0.688 | 0.672 | 0.1084 | NS | NS | NS |
| DLWG 0-12 weeks | 0.659 | 0.647 | 0.535 | 0.500 | 0.0669 | NS | ** | NS |

*P<0.05, ** P<0.01

There were no differences in the health or condition of the calves, with 6 calves from each treatment requiring electrolyte medication for scour.

**Table 2** Concentrate feed intakes (kg/head)

| | YC | | Control | | | Significance | | |
|---|---|---|---|---|---|---|---|---|
| | Male | Female | Male | Female | s.e.d | Sex | Yeast | SxY |
| Total feed intake 0-5 weeks | 26.47 | 27.50 | 23.67 | 24.34 | 2.079 | NS | NS | NS |
| Daily feed intake 0-5 weeks | 0.756 | 0.786 | 0.676 | 0.692 | 0.0599 | NS | NS | NS |
| Total feed intake 5-12 weeks | 114.81 | 112.63 | 110.21 | 105.79 | | | | |
| Daily feed intake 5-12 weeks | 2.34 | 2.29 | 2.25 | 2.16 | | | | |

*P<0.05, ** P<0.01

Based on the prices prevailing at the time of the study, feeding yeast culture cost £1.00 per calf. If the mean increase in total live weight gain (12.06kg per calf) is valued at 85p/kg then feeding a yeast culture returned a net extra income of £7.91 per calf, after deducting the cost of the higher concentrate feed intake.

**Conclusions** The results indicate that feeding a yeast culture to early-weaned calves can improve both liveweight gain and financial performance.

**Acknowledgments** Financial support from Diamond V Mills is gratefully acknowledged.

# The effect of replacement of concentrate by fodder beet on rumen fermentation and VFA production in steer cattle

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**Introduction** In a previous experiment in this series fodder beet was substituted for a major proportion of the concentrate supplement in a high concentrate diet with lactating dairy cows. The diets were offered at a restricted level of feeding and a major depression in the concentration of fat in milk was obtained with the concentrate diet (McIlmoyle *et al.,* 2001). It was the objective of the present experiment to examine the effects of diet composition, namely fodder beet versus concentrate in a silage based diet and level of feeding, namely restricted versus *ad libitum* feeding on rumen fermentation.

**Materials and methods** Six rumen fistulated steers were used in a partially balanced changeover design experiment consisting of two periods each of 18 d and three dietary treatments to determine the effects of diet on rumen fermentation. Treatment 1 (offered *ad libitum*) and 2 (offered at 2 x maintenance) consisted of grass silage and concentrate in a ratio of 30:70 (DM basis). The concentrate pellet had 222 g crude protein/kg DM and the formulation comprised (g/kg fresh weight); milled barley (130), milled wheat (310), maize gluten (140), molassed sugar beet pulp (140), soya bean meal (230), molasses (30) and mineral/vitamin supplement (20). Treatment 3 consisted of fodder beet, mineralised soyabean meal, concentrate and grass silage in the ratio 32:18:20:30 (DM basis) offered at 2 x maintenance. The composition of the grass silage was; dry matter 252 g/kg, crude protein 173 g/kg DM, ME 12.1 g/kg DM (*in vivo*) and ammonia N/total N 123 g/kg. Rumen liquor was withdrawn every hour for 24 h on days 15 and 17 of each period and analysed, with the overall mean for the 48 measurements comprising the experimental observation for a treatment. The data were subjected to analysis of variance and a t test was used to test for significance between treatment means.

**Results** The effects of dietary treatment on rumen pH, ammonia N, total volatile fatty acids (VFA) and molar proportions of VFA are shown in Table 1. The mean pH of rumen liquor was highest for the restricted fodder beet treatment (T3) compared to the restricted concentrate treatment (T2), while the *ad libitum* concentrate treatment produced the lowest pH. The fodder beet diet produced considerably lower ammonia N than the concentrate diets, while restricted feeding further increased rumen ammonia within the concentrate diets. Restricted feeding substantially reduced the concentration of total VFA, which was further reduced with fodder beet.

The acetate plus butyrate to propionate molar ratio has been described as the lipogenic:glucogenic ratio and ratios below 3.5 have been found to produce major depressions in concentration of milk fat (Oldham and Sutton, 1979). The low mean molar ratio of 3.6 observed with the concentrate diet at the restricted level of feeding is only marginally above the ratio of 3.5, and may explain the depressed concentration of butterfat which was observed with a similar restricted-fed concentrate diet in the previous study (McIlmoyle *et al.,* 2001).

**Table 1** Effect of dietary treatment on pH, ammonia N and VFA in rumen liquor

| | T1-Silage and concentrate *ad libitum* | T2-Silage and concentrate *restricted* | T3-Beet and silage *restricted* | s.e.d. | Sig. |
|---|---|---|---|---|---|
| pH | 6.18[a] | 6.32[b] | 6.52[c] | 0.04 | *** |
| Ammonia N (mg/l) | 163.3[a] | 190.3[b] | 110.3[c] | 6.07 | *** |
| Total VFA (mmol/l) | 119.3[a] | 104.8[b] | 98.5[c] | 1.86 | *** |
| *Molar ratios* | | | | | |
| Acetate:propionate | 3.4[a] | 3.1[b] | 3.8[c] | 0.07 | *** |
| (Acetate+butyrate):propionate | 4.0[a] | 3.6[b] | 4.8[c] | 0.07 | *** |

[a,b,c] Values within each row with different superscript letters were significantly different ($P<0.05$)

**Conclusions** Partial replacement of concentrate with fodder beet moderated rumen pH, depressed rumen ammonia N concentration and increased the lipogenic:glucogenic VFA ratio. While the *ad libitum* level of feeding of the concentrate diet increased the concentration of total VFA and reduced both rumen pH and ammonia N, the lipogenic:glucogenic VFA ratio was increased by the higher level of feeding.

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# Effect of feeding soya-bean meal or distillers grains on the performance of silage fed beef cattle

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**Introduction** Extracted soya-bean meal is used by many beef producers in the UK as a protein source for cattle. A number of farm assurance schemes and abattoirs now prohibit the use of this feedstuff due to the possible inclusion of genetically modified material. Organic production standards prohibit the feeding of solvent extracted feeding stuffs. Therefore, there is a requirement to evaluate alternative traceable protein sources for beef cattle. The objective of this trial was to determine the effect on cattle performance of feeding sugar beet feed with distillers grains or soya-bean meal to beef cattle on a silage based system.

**Materials and Methods** Fifty four Limousin cross Holstein bulls and heifers with a mean live weight of 290kg and 239kg respectively were used. Nine bulls and nine heifers were allocated by liveweight to the following dietary treatments in a 3x2 factorial designed experiment. The cattle were fed *ad libitum* fermented whole-crop wheat silage (DM 417g/kg, 11.6 MJ ME/kg DM, 80g CP/kg DM, 307g starch/kg DM) supplemented with either sugar beet pulp/ soya-bean meal 80:20 (S), barley distillers grains/ sugar beet pulp 50:50 (BD) or maize distillers grains/ sugar beet pulp 50:50 (MD). Supplementary feed levels were 3.8kg and 1.85kg per head per day to the bulls and heifers respectively, plus 0.2kg and 0.15kg of a high calcium beef mineral. The cattle were housed in straw-bedded pens and had free access to water and barley straw from racks. They were visually assessed for conformation using the 15 point MLC conformation and muscling score system. (1 = poor, 15 = excellent). The data was analysed using analysis of variance

**Results**. The bulls recorded significantly higher daily live weight gains, start and end weights compared to the heifers. There were no significant differences in performance between the treatments for daily liveweight gain. The mean daily liveweight gains achieved by the bulls and heifers were 1.15 and 0.84kg for the 120 day trial period.

**Table 1** Effect of diet and sex on animal performance

| | Bulls | | | Heifers | | | | Sig | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | MD | BD | S | MD | BD | S | s.e.d | Feed | Sex | Feed x Sex |
| Start weight (kg) | 294.4 | 287.0 | 288.9 | 239.1 | 233.7 | 245.2 | 6.90 | NS | *** | NS |
| End weight (kg) | 429.0 | 424.9 | 430.9 | 337.2 | 334.2 | 348.9 | 11.81 | NS | *** | NS |
| DLWG (kg, 120 days) | 1.12 | 1.14 | 1.18 | 0.82 | 0.84 | 0.86 | 0.076 | NS | *** | NS |

NS = not significant; ***P<0.001

There were no differences in the health or condition of the cattle.

**Table 2** Effect of diet on silage intake

| | Bulls | | | Heifers | | |
|---|---|---|---|---|---|---|
| | MD | BD | S | MD | BD | S |
| Silage DMI (kg/day) | 5.38 | 4.97 | 4.85 | 5.30 | 5.34 | 5.60 |

Feed costs per kg live weight gain were calculated based on the feed prices prevailing at the time of the trial (S @ £108/t, BD @ £116/t, MD @ £122/t, fermented whole-crop wheat silage @ £60/t DM) and were 63.1p, 68.9 and 74.7p for the S, BD and MD treatments for the bulls respectively. The feed costs per kg gain for the heifers were 66.6p, 68.2p, and 70.8p respectively.

**Conclusions** Feeding a 50:50 mix of distillers grains and sugar beet pulp can replace a 80:20 sugar beet pulp and soya-bean meal ration when given to silage fed beef cattle to produce similar animal and financial performance.

**Acknowledgments** Financial support from Trident Feeds is gratefully acknowledged.

## Performance of red deer grazing pure white clover or perennial ryegrass swards

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**Introduction** Farmed red deer (*Cervus elaphus*) are highly seasonal animals, showing a marked winter inappetance followed by excellent growth from turnout in spring. The quality of grazed herbage has been demonstrated to have a significant impact upon growth during the grazing season. For example, performance is improved by the provision of a sward surface height of 8 - 10 cm compared with a sward height of 5 - 6 cm (Davies *et al*, 1998). The objective of this study was to assess whether deer performance could be further enhanced by providing white clover (*Trifolium repens*) swards (C) either in early, late or throughout the grazing season compared with perennial ryegrass (*Lolium perenne*) swards (G).

**Materials and methods** Thirty-two yearling stags (mean live weight 48.4 kg) and 48 hinds (47.0 kg) were used in 1999 and 40 stags (54.6 kg) and 36 hinds (49.7 kg) were used in 2000. In both years the red deer were fed to maintain weight over the preceding winter period. Stags and hinds were allocated by liveweight to either C or G swards in a randomised block design. Animals were grazed in four paddocks with two replicates of each sward type. In mid-season (July) in both years, stags were re-randomised to either remain on the same sward type or be moved to a different sward type in a 2 x 2 factorial design. C and G swards were maintained at 8 - 10 cm throughout the study. The yearling deer were turned out to their respective pastures in April at 10 months of age and were weighed at monthly intervals up until October. All swards received 40 kg/ha P and 40 kg/ha K dressing in February and the G swards received 150 kg/ha N in three equal applications over the season. Live weight data were analysed by analysis of variance.

**Results** The daily liveweight gain in yearling stags and hinds on the two sward types are shown in Table 1. Yearling stags and hinds grazed on C swards performed significantly better overall than those grazed on G swards in both years (mean +53% and +35% for stags and hinds respectively). This superior performance on C swards was most marked in late season when growth responses were +103% and +45%, whereas early season responses were smaller at +29% and +24% for stags and hinds respectively.

**Table 1** Daily liveweight gain (g) of yearling red deer hinds and stags on perennial rye grass and white clover swards

| Treatment | Hinds | | | | Stags | | | |
|---|---|---|---|---|---|---|---|---|
| | April - July | | April - October | | April - July | | April - October | |
| | 1999 | 2000 | 1999 | 2000 | 1999 | 2000 | 1999 | 2000 |
| C | 243 | 192 | 209 | 160 | 367 | 253 | 271 | 222 |
| G | 202 | 150 | 175 | 107 | 270 | 206 | 187 | 121 |
| s.e.d | 7.5 | 17.6 | 5.6 | 7.9 | 19.1 | 15.5 | 13.1 | 18.0 |
| significance | *** | * | *** | *** | *** | ** | *** | *** |

Growth rates of stags (g/day) from July to October were 217, 114, 143 and 214 (s.e.d 21.7) in 1999 and 207, 72, 101 and 206 (s.e.d 16.1) in 2000 for CC, CG, GG and GC respectively. Growth rates on clover in late season were unaffected by the early season sward type, whereas CG stags tended to perform less well than GG stags in late season. Overall deer performance in 2000 was inferior to that in 1999 due to a later turnout date and poor climatic conditions. Mean crude protein content (g/kg DM) of the herbage for both years was 242 for C and 147 for G swards.

**Conclusions** White clover swards resulted in significantly better performance of yearling red deer stags and hinds during 10-16 months of age while at pasture. This was particularly apparent after July, when grass quality had begun to deteriorate. The higher crude protein content of C swards may have contributed to this, although herbage intakes may be more important. The judicious use of pure white clover swards during the grazing season could enable the deer producer to optimise growth rates, enhance marketing opportunities from pasture and reduce the need for expensive winter feeding.

**Acknowledgements** This work was funded by the Department of Environment, Food and Rural Affairs

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# Effect of energy source or fibre level in concentrates fed to finishing Swaledale lambs.

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**Introduction**. Concentrate finishing systems for store lambs often involves a dramatic change in the basal diet as lambs are moved from being managed on, e.g. grass and / or roots to concentrates. If this is done too quickly there is a risk of digestive disturbances leading to acidosis and secondary infections. Hence, the selection of the carbohydrate balance between starch and digestible fibre is crucial as ruminants fed high levels of starch-based concentrates can develop sub-clinical acidosis and liver abscess, leading to decreased voluntary food intake and daily live weight gain (DLWG). The aim of this study was to evaluate two concentrate formulations for effective growth for finishing of Swaledale lambs, one concentrate being starch biased the other digestible fibre biased.

**Materials and Methods.** 160 Swaledale lambs were divided into 16 pens of 10, balanced for initial lamb weight, housed in the same building with *ad-libitum* access to water and barley straw. The 16 pens were allocated randomly to one of two iso energy (ME) and iso protein diets – high digestible fibre (HDF) biased e.g molassed sugar beet pulp, 86.2% dry matter, 16% protein, 10.7% fibre 9.2% ash and 4.8% oil or the starch biased, e.g. wheat diet (S), 86.2% dry matter, 16% protein, 8.9% fibre, 8.1% ash and 4.8% oil. Concentrate intakes were recorded on a weekly, feed weigh backs fortnightly, lambs weight fortnightly. Lambs were assessed for finish (fat class 3L). Data was analysed using Minitab 13.1.

**Results.** The physical, carcass and financial performance data are presented in tables 1 and 2. These show a significant benefit ($p<0.05$) in terms of FCE for the starch biased feeding regime.

**Table 1:** Physical performance of Swaledale lambs fed either a starch or digestible fibre diet

| | HDF | S | s.e.d. | Significance |
|---|---|---|---|---|
| Start Weight (Kg) | 27.45 | 27.35 | 0.28 | NS |
| Live weight (Kg) | 34.61 | 34.74 | 0.40 | NS |
| Days on Trial | 61.47 | 59.51 | 2.36 | NS |
| Live weight Gain (Kg) | 7.05 | 7.25 | 0.18 | NS |
| Daily Live weight Gain (g/d) | 122.30 | 124.90 | 0.01 | NS |
| FCE | 0.07 | 0.08 | 0.004 | * |
| £/Kg Gain | 1.86 | 1.67 | 0.09 | * |
| Total Feed Intake/pen (Kg) | 912.10 | 860.70 | 54.30 | NS |

* $p\leq0.05$

**Table 2:** Carcass characteristics of Swaledale lambs fed either a starch or digestible fibre diet

| | HDF | S | s.e.d. | Significance |
|---|---|---|---|---|
| Carcass weight (Kg) | 15.25 | 15.19 | 0.21 | NS |
| KO value | 44.10 | 43.70 | 0.52 | NS |
| Conformation | 2.25 | 2.31 | 0.06 | NS |
| Fat | 2.81 | 2.82 | 0.06 | NS |
| Value (£) | 31.32 | 30.74 | 1.05 | NS |

There were no major health differences between groups.

**Conclusion** The results show that feeding Swaledale lambs a starch biased lamb pellet, using the costs prevailing at the time, is more cost effective compared to feeding a digestible fibre biased lamb pellet. However, these lambs had previously been fed a grass based diet and the concentrates had been introduced gradually over a 10 day period. The benefits of the starch biased feeds may not be seen if the lambs had been changed over quickly. Store lamb finishing recommendations must consider the total feeding management.

**Acknowledgements** Newton Rigg are grateful to ABNA Ltd. for the financial funding received.

# The effect of live yeast (Yeasacc) and yeast culture (Diamond V XP) on the performance of intensively fed Swaledale store lambs

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**Introduction**. Concentrate finishing systems for store lambs often involves a dramatic change in the basal diet as lambs are moved from being managed on, e.g. grass and / or roots to concentrates. If this is done too quickly there is a risk of digestive disturbances leading to acidosis and secondary infections. However, the desire of the lamb finisher is to transfer the lambs onto the concentrate diets as quickly as possible and reduce rumen friendly materials such as straw to maximise performance. Yeast products have been shown repeatedly to reduce the production of lactic acid and stimulate the utilisation of lactic acid by certain rumen microflora, e.g. *M. elsdenii* leading to a more optimal rumen environment for the rumen microflora that in turn enhances nutrient supply and animal performance.
The aim of this trial was to determine whether there would be any response to the inclusion of two yeast products, a live yeast, Yeasacc 1026 and a yeast culture, Diamond V XP, in intensively finished Swaledale wether store lambs.

**Materials and methods** 96 Swaledale wether lambs were used for this study, they were grouped into 3 diets balanced for lamb weight. The three diets (based on maize gluten, molassed sugar beet pulp, wheat, sunflower extract, linseed and molasses) were introduced gradually over a two-week period and then fed *ad-libitum* pellets plus *ad-libitum* straw and water. The three diets were 1 – Control (16 % protein Store Lamb Finisher), diet 2 – Control + Yeasacc at 1Kg/tonne, and diet 3 – Control + Diamond V XP at 5 Kg/tonne. Feed intakes, lamb weight and assessment for finish were measured fortnightly. All lambs were weighed on selection for slaughter, sold deadweight and carcass information was collected.

**Results** Table 1: *Table showing performance parameters measured*

| | Control | + Yeasacc | + Diamond XVP | s.e.d. | Significance |
|---|---|---|---|---|---|
| Start Weight (Kg) | 22.5 | 24.5 | 24.1 | 0.674 | ** |
| Live Weight (Kg) | 34.5 | 34.9 | 34.9 | 0.459 | NS |
| Carcass Weight (Kg) | 14.8 | 14.8 | 14.6 | 13.406 | NS |
| Killing out Value | 42.8 | 42.5 | 41.7 | 1.315 | NS |
| Weight Gain (Kg) | 12.3 | 10.4 | 10.8 | 0.741 | NS |
| DLWG (Kg) | 0.236 | 0.218 | 0.214 | 0.004 | ** |
| Conformation | 2 | 2 | 1.88 | N/a | NS |
| Fat | 2.76 | 2.66 | 2.56 | 0.139 | NS |
| £/Kg | 1.77 | 1.73 | 1.73 | 0.021 | NS |
| £/lamb | 26.1 | 25.7 | 25.2 | 1.015 | NS |
| Days on trial | 52.21 | 48.6 | 51.44 | 3.207 | NS |

*Significance ** $p<0.01$, conformation graded as follows – E=5, U=4, R=3, O=2, P=1, Fat class – 1=1, 2=2, 3L=2.8,, 3H=3.2, 4L=3.8, 4H=4.2, 5=5*

From the above table it can be seen that the inclusion of yeast products had no effect on lamb performance, with the only significant difference being for DLWG where lambs fed the control performed better than those containing yeast products. However these lambs were originally lighter than those on the other two diets suggesting that these lambs could have under gone a higher proportion of compensatory growth.

**Conclusion.** The inclusion of these two yeast products had no effect on Swaledale lamb finishing performance. However, as the mode of action of yeast products is recognised to be via a change in the rumen environment it could be that the finishing period for store lambs is insufficient for this benefit to be manifested. The use of yeast products in early lamb finishing systems may be more appropriate.

**Acknowledgements.** Newton Rigg are grateful for the funding received from ABNA limited

# *n*-alkane concentration in semi-natural plant species found in upland areas of the UK

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**Introduction** The *n*-alkane technique (Dove and Mayes, 1991; Mayes and Dove, 2000) has been developed as a method to determine the voluntary intake of herbivores grazing freely at pasture. The technique relies on the fact that each herbage species contain a unique profile of odd-chain *n*-alkane compounds within the cuticular waxes of each plant part. Whilst some *n*-alkane profiles for the major intensively managed herbage species are available, there is little comprehensive information on the *n*-alkane profiles of many of the herbage species found commonly in semi-natural vegetation communities (SNVC) in the hills and uplands of the UK. If the *n*-alkane technique is to be extended for use with herbivores grazing SNVC's, it is necessary that such profiles be determined. This study details the *n*-alkane concentrations found in ten herbage species sampled from SNVC's in the UK during late summer in 2000.

**Materials and methods** Samples of the most abundant semi-natural plant species found in *Molinia* dominated SNVC upland pasture at ADAS-Redesdale (RED) and in *Nardus* dominated SNVC upland pasture at ADAS-Pwllpeiran (PWL) were harvested by hand plucking in late September 2000. Triplicate samples (approximately 1200 g fresh weight) of *Molinia caerulea* (MC), *Nardus stricta* (NS), *Calluna vulgaris* (CV), *Juncus effusus* (JE), *Eriophorum vaginatum* (EV) and *Erica tetralix* (ET) were obtained from RED. In addition, duplicate samples of MC, NS, CV, JE, *Eriophorum angustifolium* (EA), *Vaccinium myrtillus* (VM), *Juncus squarosus* (JS) and a mixture of undefined fine grasses (GRA) were obtained from PWL. Herbage samples were held overnight at 4°C and then transfered on ice to the laboratory, freeze dried, milled through a 1 mm screen and subsequently analysed for *n*-alkane concentrations by Gas Liquid Chromotograph. Where species were present in both locations the significance of species and location as sources of variation in the data set were tested using the residual maximum likelihood facility (REML) in Genstat 5.

**Results** Average (+/- s.e.) *n*-alkane concentrations for all the species of herbage collected from both RED and PWL are given in Table 1. No C28, C35 or C36 *n*-alkane were found in any of the herbage species studied. Low total *n*-alkane concentrations (mg/kg DM) were seen for both MC (107) and EA (66) whereas relatively high total values were recorded for CV (1234), EV (818) and ET (1503). Average total *n*-alkane concentration for the remaining herbage species were within the range 370-486 mg/kg DM. Individual *n*-alkane concentrations varied between herbage species, particularly for the most abundant C29, C31 and C33 *n*-alkanes. In the main, both herbage species, location and the interaction between them were significant sources of variation in *n*-alkane concentration between samples (Table 2).

**Table 1**. Mean (+/- s.e.) *n*-alkane concentration (mg/kg DM) of ten upland herbage species sampled in the UK.

| *n*-alkane | MC | NS | CV | JE | EV | ET | EA | VM | JS | GRA |
|---|---|---|---|---|---|---|---|---|---|---|
| C27 | 25 (3.0) | - | 33 (4.8) | - | - | 57 (5.2) | 34 (9.5) | 46 (4.0) | - | - |
| C29 | 53 (7.8) | 138 (6.7) | 96 (10.6) | 272 (27.2) | 117 (14.7) | 159 (2.6) | 22 (2.0) | 143 (9.0) | 42 (2.5) | 162 (88.5) |
| C30 | - | - | - | - | - | 22 (0.9) | - | - | - | - |
| C31 | 29 (10.2) | 181 (19.0) | 576 (65.5) | 117 (15.7) | 500 (9.5) | 891(19.5) | 11(11.0) | 228 (13.0) | 354 (48.0) | 260 (126.0) |
| C32 | - | - | 46 (4.0) | - | - | 36 (1.2) | - | - | - | - |
| C33 | - | 51 (7.5) | 475 (49.0) | - | 201 (4.4) | 338 (21.9) | - | 34 (1.0) | 64 (13.0) | 49 (34.0) |
| Total | 107 (20.7) | 370 (25.3) | 1234 (136) | 394 (39.6) | 818 (24.6) | 1503 (35) | 66 (16.5) | 451 (27.0) | 460 (63.5) | 486 (264) |

**Table 2**. *n*-alkane concentrations (mg/kg DM) of four upland herbage species (S) sampled from two locations (L).

| | | | | | | | Significance of effects | | |
|---|---|---|---|---|---|---|---|---|---|
| *n*-alkane | | MC | NS | CV | JE | s.e.d | S | L | SxL |
| C29 | RED | $41^{e}$ | $142^{c}$ | $82^{de}$ | $309^{a}$ | 21.0 | *** | NS | *** |
| | PWL | $70^{de}$ | $132^{c}$ | $116^{cd}$ | $217^{b}$ | | | | |
| C31 | RED | $15^{f}$ | $152^{cd}$ | $478^{b}$ | $119^{de}$ | 36.6 | *** | *** | *** |
| | PWL | $50^{ef}$ | $226^{c}$ | $724^{a}$ | $113^{de}$ | | | | |
| Total | RED | $76^{d}$ | $333^{c}$ | $1026^{b}$ | $429^{c}$ | 67.1 | *** | *** | *** |
| | PWL | $154^{d}$ | $410^{c}$ | $1546^{a}$ | $330^{c}$ | | | | |

Within each parameter, values not sharing common superscripts differ significantly ($P<0.05$).

**Conclusions** Significant differences exist in both the total *n*-alkane concentration and in the pattern of odd-chained *n*-alkane content between the herbage species sampled. Practically, use of the *n*-alkane technique should allow a variety of voluntary intake and diet selection studies to be carried out with herbivores grazing freely on SNVC pastures.

**Acknowledgements** This work was funded by DEFRA.

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# Early lactation responses to red clover or ryegrass silages offered to dairy cows during the dry period

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**Introduction** Energy and protein supply during the dry period can affect subsequent milk production and composition (Moorby *et al*., 1996). Grass silage is a common ingredient of dry cow diets, but although it is usually adequate in crude protein concentration (CP), a high proportion is frequently in non-protein forms. Red clover silage has shown interesting characteristics that would increase true protein supply to ruminants (Broderick *et al*., 2000), which could avoid the use of more expensive concentrate supplements. The objective of this experiment was to compare red clover and ryegrass silage when fed as the sole source of forage to dry cows with a diet comprising ryegrass silage and a protein supplement.

**Material and methods** Forty five multiparous Holstein-Friesian dairy cows were allocated to one of three experimental diets. The experimental diets were red clover silage (RC), ryegrass silage (RG), and a TMR composed by RG + soyabean (SO; formulated to be isonitrogenous with RC). All diets were individually offered *ad libitum* for six weeks before calving. After calving, all animals were individually offered *ad libitum* a standard diet composed of RG, rolled barley grain and soya bean meal (Lact). Live weight (LW) and condition score (0-5 CS scale) were measured weekly; dry matter intake (DMI) was estimated on a daily basis for the whole experimental period. Calf birth weight (CW) was recorded within 24 hours of birth, milk production and composition were assessed weekly during the first three weeks of lactation and analysed by NMR. Results were analysed by one-way analysis of variance; and means were compared by least significant difference.

**Table 1**. Chemical composition of feeds. Means in g/kg freeze dry matter (FDM), unless otherwise stated.

| | RC | RG | SO | Lact |
|---|---|---|---|---|
| FDM (g/kg fresh matter) | 231 | 198 | 223 | 285 |
| Acid detergent fibre | 364 | 375 | 337 | 240 |
| Crude protein | 197 | 158 | 208 | 208 |

**Table 2**. Maximum live weight (Max LW) dry matter intake (DMI), milk yield and milk component contents and yield for dairy cows offered during the dry period with RC, and SO.

| | RC | RG | SO | SEM[1] |
|---|---|---|---|---|
| Max LW (kg)[†] | $735^{b}$ | $730^{b}$ | $747^{a}$ | 5.2* |
| DMI pre-calving (kg/day)[†] | $14.0^{a}$ | $12.0^{b}$ | $12.6^{b}$ | 0.38*** |
| DMI post-calving (kg/day)[†] | 16.0 | 15.9 | 17.2 | $0.51^{ns}$ |
| Milk yield (kg/day) | 29.5 | 30.0 | 32.8 | $1.18^{ns}$ |
| Protein yield (g/day) | $1084^{b}$ | $1131^{ab}$ | $1208^{a}$ | 34.6* |
| Fat yield (g/day) | 1320 | 1418 | 1352 | $58.6^{ns}$ |
| Lactose yield (g/day) | $1491^{b}$ | $1512^{b}$ | $1668^{a}$ | 53.2* |
| Protein (g/kg) | 34.0 | 34.6 | 34.5 | $0.5^{ns}$ |
| Fat (g/kg) | 41.3 | 43.9 | 38.6 | $1.7^{ns}$ |
| Lactose (g/kg) | $46.5^{ab}$ | $45.9^{b}$ | $47.4^{a}$ | 0.34** |

[1] Standard error of the mean. *, *P = 0.05*; **, *P = 0.01*. Means within the same row with different superscripts differ (P = *0.05*).
[†] Covariable (initial LW) corrected means.

**Results** Chemical composition of the diets are shown in Table 1. Although RC and SO were formulated to be isonitrogenous, SO was slightly higher in CP concentration. Maximum CS (Max CS, corrected by mean initial CS = 2.4), LW gain (general mean = 1758 g/day) and CW (general mean corrected by initial LW = 42.3 kg) were not affected by any treatment. Maximum LW (Max LW, corrected by initial LW) was higher for SO than for RG or RC. During the pre-calving period, DMI was higher for RC than for the other two treatments, but this difference disappeared after calving. Milk lactose concentration was higher for SO with respect to RG. Lactose yield was significantly increased by SO treatment with respect to RC and RG. Protein yield was significantly higher for SO with respect to RC. Milk yield was also higher for SO than for RC (contrast comparison, *P = 0.05*).

**Conclusion** Dairy cows offered red clover silage during the dry period ate more than those offered the two other diets based on ryegrass silage. Red clover silage diet produced higher pre-calving dry matter and crude protein intake, but lactational performance during the first three weeks post-calving did not show differences between red clover and ryegrass silage diets. At a similar CP concentration in prepartum diets, cows previously offered SO performed better than those offered RC in results of milk yield, and milk lactose and protein yield.

**Acknowledgements** This work was funded by the Ministry of Agriculture, Fisheries and Food. G. Jaurena is grateful for scholarships provided by the British Council and Fundación Antorchas (Argentina).

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# Effects of behaviour selection on litter size, fetal development and plasma progesterone concentrations during pregnancy in silver fox vixens

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**Introduction** Animal domestication is a natural selection experiment the important result of which is a great increase in the rate of appearance of new forms and in the wild range of variation of organisms. Analysing different aspects of this problem, D.K. Belyaev has came to a hypothesis that the morphological and physiological reorganisation of domestic animals has been going by the way of unconscious selection of animals on their behaviour, carried out by person at the very first stages of domestication (Belyaev, 1979). To testify this hypothesis, a population of tame silver foxes has been produced in long-term selection for lack of aggression and fear towards humans (domestic behaviour) at the Institute of Cytology and Genetics in Novosibirsk, Russia. In the process of selection the genetic transformation of behaviour and morphology, and physiological functions has been observed (Trut, 1999). In particular, selected animals show no aggressiveness to man, behave amicably towards humans and have some changes in the coat colour and body constitution (Trut, 1999). The important part of Belyaev's hypothesis was the assumption that selection for domestic behaviour could affect the reproductive function, in particular the pituitary-gonadal axis controlling reproduction and fertility. The aim of this study was to obtain information about possible changes in reproduction between control (C) and domesticated (D) vixens. Reproductive performance, potential fertility, embryonic mortality and fetal viability were analysed for vixens from domesticated and control population. In addition, plasma progesterone concentrations were determined in selected and control females during pregnancy.

**Materials and methods** The animals used in this study were silver fox vixens (*Vulpes vulpes*) from control and selected populations maintained at the Experimental Animal Farm of the Institute of Cytology and Genetics of the Siberian Department of the RAS. The method of fox selection for domestic behaviour has been described in details (Trut, 1995). Litter size after birth was recorded in fox vixens for a five-year period (D: n=405, C: n=1220). Pregnant females (D: n=44, C: n=48) were sacrificed at different stages after implantation and the number of corpora lutea and implantation sites were recorded. The number of viable fetuses was recorded for each vixen, and each fetus was weighed. To determine plasma progesterone concentrations in pregnant foxes, the blood samples were taken from *v. saphena* every 5-10 days during pregnancy (D: n=13, C: n=12). Progesterone was determined by RIA using commercial kits (*Cea-Ire-Sorin,* France). Steroid was extracted from the plasma samples by freshly redistill ethyl ether. The reproductive and hormonal data were statistically analysed using analysis of variance and Student's t-test.

**Results** The litter size was higher in domesticated females as compared with control females (D: 5.4±0.1, C: 4.5±0.1; $P<0.05$). Domesticated foxes ovulated more egg cells and formed larger number of corpora lutea than control foxes (D: 7.3±0.2, C: 6.4±0.2, $P<0.05$). The embryonic mortality was the same in both behavioural groups (D: 2.6±0.4, C: 2.0±0.3). A significant positive correlation existed between the number of corpora lutea and implantation sites ($r=0.45$; $P<0.05$) only in the control foxes. The fetal weight was smaller in domesticated group than in control on day 45 of pregnancy (D: 50.6±1.3 g, n=58; C: 59.7±1.2 g, n=58, $P<0.05$) and on day 50 of pregnancy (D: 85.5±2.5 g, n=37; C: 94.8±3.0 g, n=30, $P<0.05$). The coefficient of correlation between fetal and mother weight was significant on day 50 of pregnancy in both groups (D: $r=0.80$, C: $r=0.92$, $P<0.05$). The positive relationship was revealed between fetal weight and the number of fetuses on day 50 of pregnancy in control vixens ($r=0.88$; $P<0.05$), while it was negative in domesticated vixens ($r=-0.45$; $P<0.05$). The progesterone plasma levels during the preimplantation period of pregnancy were significantly higher in domesticated vixens than in control vixens (day 2 after mating D: 11.90±0.88 ng/ml, C: 8.00±0.95 ng/ml, $P<0.05$; day 5 after mating D: 21.30±1.05 ng/ml, C: 18.15±0.83 ng/ml, $P<0.05$).

**Conclusions** The data obtained suggest that the reproductive potential increased in domesticated females compared with control females and this increase was due to the higher number of corpora lutea. The increased progesterone levels during early pregnancy in domesticated vixens may be a part of the hormonal mechanism involved in the increased reproductive potential attained by selection for domestic behaviour.

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# Influence of Genetic Merit on Fertility Traits of Dairy Cattle on Commercial Farms

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**Introduction** The selection of dairy cattle for high milk production is thought to be linked to increased problems with fertility. Most research has concentrated on the influence of actual yields rather than the effect of genetic merit on fertility. It has also been reported that by maintaining a high level of herd management the reproductive efficiency problems associated with high milk production can be removed (Nebel and Gilliard, 1993). The current work estimated the influence of genetic merit (defined as PIN95), 90-day daily milk yield, deviation from herd mean 305-day milk yield and body condition score on fertility, in an attempt to assess whether actual yield has more effect than potential yield as indicated by genetic merit.

**Materials and Methods** Fertility records from 874 cows (with confirmed pregnancy diagnosis) from herds in Kent were used to estimate the influence of PIN95 and milk production on fertility traits.

Model 1 Fertility trait = YOC + MOC + P + H + A

Where Fertility traits included days from calving to first service (C-S), days from first to successful service (S-S), interval from calving to conception (C-C) and services per conception (S/C). YOC is year of calving, MOC is month of calving, P is parity, H is the herd (defined in terms of average 305-day milk yield) and A is either 90-day daily yield, 90-day body condition score, deviation from herd average 305-day milk yield or PIN95.

**Table 1:** Estimates of the influence of PIN95, deviation from herd 305-day milk yield, 90-day milk yield and 90-day condition score on days calving to first service (C-S), first to successful service (S-S), calving to conception (C-C) and services per conception (S/C) on commercial farms

| Fertility Trait | PIN95 | | | Deviation from herd 305-day Milk Yield (kg) | | |
|---|---|---|---|---|---|---|
| | b | se | p-value | b | se | p-value |
| C-S (d) | 0.14 | 0.006 | 0.0332 | 0.005 | 0.0009 | 0.0001 |
| S-S (d) | -0.05 | 0.103 | n.s. | 0.004 | 0.0015 | 0.0063 |
| C-C (d) | 0.14 | 0.117 | n.s. | 0.010 | 0.0017 | 0.0001 |
| S/C | 0.003 | 0.0018 | n.s. | 0.0001 | 0.00003 | 0.0001 |
| | 90-day daily milk yield (kg/d) | | | 90-day BCS | | |
| | b | se | p-value | b | se | p-value |
| C-S (d) | 0.45 | 0.221 | 0.0400 | -9.78 | 3.142 | 0.0019 |
| S-S (d) | 0.21 | 0.347 | n.s. | -4.92 | 4.885 | n.s. |
| C-C (d) | 0.59 | 0.395 | n.s. | -17.05 | 5.405 | 0.0017 |
| S/C | 0.007 | 0.0063 | n.s. | -0.095 | 0.088 | n.s. |

**Results** The results (Table 1) show that the relationship between 305-day milk yield and fertility traits was more highly significant than either the relationship between genetic merit or 90-day milk yield and fertility. Both PIN95 and 90-day milk yield significantly increased the number of days to first service (C-S), (0.14d/PIN95 and 0.45d/kg milk/d, respectively), this increase did not follow through to, days calving to conception (C-C). A positive deviation from herd mean 305-day milk yield significantly reduced reproductive efficiency. Increasing 90-day condition score resulted in a decrease in the intervals from calving to first and successful services. The results also showed that the influence of herd 305-day milk yield on fertility was more significant than either PIN95, 90-day milk yield or deviation from herd 305-day milk yield. Figure 1 shows the increase in C-C and S/C with herd mean milk yield, and represents a between herd comparison, while results for deviation from herd 305-day milk yield represent a within herd comparison.

**Conclusions** The significant influence of PIN95 on days calving to first service meant that higher genetic merit animals had longer intervals to first service. Positive deviations from herd mean 305-day milk yield led to a reduction in reproductive efficiency of dairy cattle on commercial farms. The significant influence of 90-day BCS on C-S and C-C indicates that body condition score at around the time of re-breeding is more important than 90-day milk yield or PIN95 for fertility traits on commercial farms. Animals in better condition had less days from calving to first service and from calving to conception.

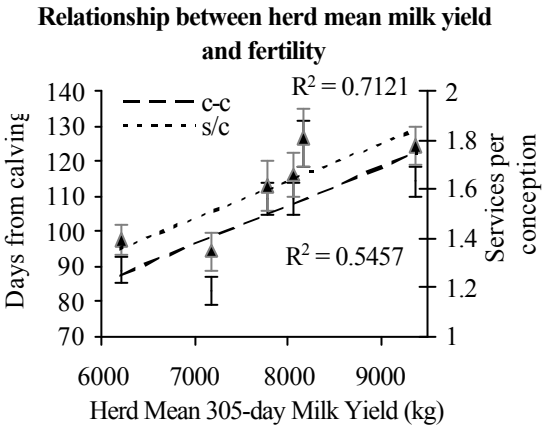


**Figure 1:** Relationship between herd (mean 305-day milk yield) and days calving to conception (C-C), and services per conception (C/S)

**Acknowledgements** The authors wish to thank the South of England Agricultural Society and Wye College, University of London for financial support, and Philip Drury for help with the collection of data.

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# Pattern of follicular growth and ovulation frequency in post-partum beef cows after a temporal calf removal associated with a gonadotrophin release hormone

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**Introduction** The main cause of prolonged post-partum anoestrous in suckled cows is the failure of dominant follicles to ovulate. Increased LH pulse frequency is necessary to promote dominant follicles (DF) to ovulate. A 96 h period of calf removal lead to a 2-fold increase in the LH pulse frequency in the study of Stagg *et al*. (1998). Moreover, acute calf isolation and once-a-day suckling shortly after the emergence of the fourth follicular wave induced two thirds of cows to ovulate the DF of that wave (Sinclair *et al*., 1999). The present study was carried out to investigate the effect of 96 h calf removal and GnRH administration on the duration of the post-partum anoestrous period in suckled beef cows.

**Materials and methods** Two experiments were done in this study. Experiment 1 involved twenty multiparous Hereford cows that calved with (mean ±sem) 355±32.7 kg and 4.0±0.4 units of body condition score (BCS) (scale 1 to 8, Earle, 1976). The cows remained with their calves until 61.4±7.4 days *post-partum,* when they were assigned to two treatments (day 0): in one group each calf was present with unlimited contact and access to its dam (C; n=10); in the second group each calf was removed from its dam from a period of 96 h (CR, n=10). One cow from the treated group had ovulated before the treatment was applied and was eliminated from the analysis of the data. Experiment 2 involved twenty four multiparous Hereford cows that calved with 383 ±34.5 kg and with a BCS of 3.9±0.1 units. The cows remained with their calves until 54.4±0.8 days *post partum*, when they were assigned to two treatments (day 0): in one group each calf was completely removed from its dam for a period of 96 h (CR, n=12); in the second group in addition to the 96 h of temporary weaning, a dose of 250µg of GnRH was administered to the cows the day before calf return (CR+GnRH, n=12). In both experiments all types of contact (visual, olfactory and audible) were avoided during this period. Follicle growth was monitored daily from 10 days before calf removal until 10 days after the calves returned with their dams (24 days of scanning period) using transrectal ovarian ultrasonography. Blood samples were collected twice a week for progesterone analysis. Continuous variables were analysed by ANOVA in the GLM procedure and frequencies by Fisher´s exact test.

**Results** In Experiment 1, in the CR group (n=9) maximum diameter attained by the dominant follicle was larger after (11.8 ±0.5 mm) than before (10.4± 0.2mm) calf removal (P<0.001). Number of cows that ovulated within a period of 12 days after CR was higher in CR group (3/9) compared with the C group (0/10; P<0.05). The interval from CR to ovulation was 9±1.8 days for cows in CR treatment. In Experiment 2, the administration of GnRH together with a 96 h calf removal induced all 12 cows to ovulate in contrast to only 4/12 cows that did not receive GnRH (Table 1). The diameter of the follicle promoted to ovulate tended to be smaller in CR+GnRH cows (9.8±0.3 mm) than in CR cows (11.3±0.9 mm, P=0.06). Similarly, the maximum diameter attained by the corpus luteum (CL) tended to be smaller in cows of the CR+GnRH group compared with those in the CR group (12.1±2.4 *v.* 16.7±7.5 mm respectively, P=0.08). Consequently, the maximum progesterone concentration produced by the CL within 14 days after CR tended to be lower in CR+GnRH cows than in CR cows (0.66± 0.1 *v.* 2.00±1.1 ng/ml, respectively; P=0.06).

**Table 1** Follicular growth and reproductive performance in CR and CR+GnRH cows (experiment 2)

| | CR | CR+GnRH |
|---|---|---|
| n | 12 | 12 |
| Maximum diameter of follicle before CR (mm) | 10.4±0.4 | 10.0±0.3 |
| Maximum diameter of follicle after CR (mm) | 11.3±0.4 | 10.1±0.3 |
| Number of cows ovulating after CR (within 12 days) | 4/12 [a] | 12/12 [b] |
| **Interval from calf removal to ovulation (days)** | **5.8±0.8[a]** | **1.8±0.1[b]** |
| Maximum diameter of the ovulatory follicle (mm) | 11.3± 0.9 | 9.8 ±0.3 |
| Number of cows with short oestrous cycles (<17 days) | 4/4[a] | 12/12[b] |
| Number of cyclic cows at the end of the mating period | 5/12 | 4/12 |

a v b: P<0.05

**Conclusions** Calf removal for 96 h at d 60 post partum induced ovulation in almost 35 % of cows in low to moderate body condition at calving. Calf removal for 96 h together with GnRH administration induced all cows to ovulate, being more effective than CR alone. However cows were unable to restore normal oestrous cycles. The smaller ovulatory follicle lead to a smaller CL formed after the treatment which would not be able to produce enough progesterone to exert the priming effect on the hypothalamus-pituitary axis as well as the regulation of uterine receptors.

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# Measurement of milk progesterone using near infrared spectroscopy

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**Introduction** It is now well established that milk progesterone analysis can provide a valuable tool in reproductive management of the dairy cow. With the advent of relatively cheap cow side milk progesterone tests, the principle block to widespread monitoring is the time and effort involved in the actual collection and analysis of the milk samples. This has led to a research drive to develop on line milk progesterone monitoring systems whereby progesterone is routinely monitored at normal milking. Near-infrared spectroscopy (NIRS) is widely used in the agricultural industry for analysing cereals and forages and is used for on-line testing in the food and pharmaceutical industries. Vibrations of chemical bonds between carbon, oxygen, hydrogen and nitrogen atoms affect the amount of near-infrared light absorbed at individual wavelengths, so different molecules show unique patterns in the spectrum. These patterns are related to determined concentrations of chemicals in calibration samples to develop equations that predict concentrations in unknown samples. This project set out to assess the use of this technique to measure milk progesterone.

**Materials and methods** In all studies, samples were analysed using a near-infrared scanning monochromator (model 6500; NIRSystems, Silver Spring, MD). Reference milk progesterone concentrations were determined using a commercially available ELISA kit (Ridgeway Scientific). In Study 1, 100 fresh milk samples were scanned onto the NIRS and reference progesterone concentrations determined by ELISA to compare the two measurement systems. In Study 2 additional progesterone was added to a milk sample with an initial concentration of 4ng/ml to achieve concentrations of 9 and 24ng/ml. Aliquots of milk containing the 3 different concentrations of progesterone were then subjected to ELISA measurement and NIRS prediction to determine the precision with which the additional progesterone could be detected. In Study 3, 44 milk samples were collected from cows during the first 2 weeks post partum, prior to any anticipated luteal activity, and subjected to ELISA measurement and NIRS prediction to determine the accuracy of NIRS prediction based on samples with low progesterone concentrations. In Study 4, milk samples were collected from 5 cows at 30 second intervals throughout milking to monitor changes in progesterone concentration that occur during the course of milking. In Study 5 the degree to which milk fat protein and lactose interfere with milk progesterone measurement was determined. Fat, crude protein and lactose concentrations of milk were determined by NIRS calibrated using the Babcock method for fat (AOAC, 1990 - method 989.04 AOAC; Official Methods of Analysis, Association of Official Analytical Chemists, Arlington, Virginia, 1990), an elemental N analyser (NA 2000, Fisons Instruments, Crawley, Sussex) and test kits for lactose (Boehringer Mannheim, Lewes, Sussex).

**Results** In Study 1, ELISA progesterone measurements demonstrated samples with milk progesterone concentrations covering the whole physiological range (0.5 to 60ng/ml). Based on this wide range of concentrations NIRS predicted progesterone concentration with coefficient of correlation (R) of 0.94 and a slope of 1.01 showing that progesterone measurements were similar between the two methods. However, the standard error of prediction (the accuracy of the NIRS prediction of progesterone concentration) was ±3.9ng/ml demonstrating a lack of precision. In Study 2, in which additional progesterone was added to a milk sample, NIRS predicted milk progesterone concentration with an R of 0.99 and a slope of 0.99 demonstrating similar recovery of progesterone to the ELISA measurements. However, the standard error of prediction was once again rather imprecise (±2.5ng/ml). In Study 3, of the 44 samples assayed by ELISA 3 samples contained high progesterone (> 3ng/ml) and were excluded from NIRS calibration. Based on the remaining 41 samples, NIRS predicted progesterone concentration with an R of 0.95 and a standard error of prediction of only ±0.3ng/ml. However, the accuracy with which the prediction equation based on low samples could predict progesterone in samples with higher progesterone was very poor (e.g. ELISA 12ng/ml, NIRS 1.8ng/ml; ELISA 6ng/ml, NIRS 0.5ng/ml). In Study 4, collection of milk samples throughout milking revealed that both ELISA measurement and NIRS prediction of milk progesterone showed a similar pattern with progesterone concentration rising over the first 90 seconds of milking and then remaining relatively constant for the remainder of the milking period. In Study 5, NIRS gave excellent predictions of both milk fat (R 0.99, slope 0.98, standard error of prediction ±0.08%) and milk protein (R 0.99, slope 1.01, standard error of prediction ±0.04%) compared with much more complicated laboratory analysis techniques. NIRS also gave a reasonable prediction of lactose content though prediction of lactose showed considerably more variability (R 0.82, slope 1.01, standard error of prediction ±0.12%). There was no relationship between lactose or protein and ELISA measurement or NIRS prediction of milk progesterone. However, increasing milk fat was associated with higher ELISA measurement (R = 0.53) and NIRS prediction (R = 0.52) of milk progesterone.

**Conclusions** The results demonstrate that while NIRS does go some way towards predicting milk progesterone concentrations, the precision with which this is currently achieved is not sufficient to make it a practical means of assessing milk progesterone in an on-line detection system.

**Acknowledgements** This work was supported by the Milk Development Council.

# Influence of plasma urea nitrogen on superovulatory response and embryo recovery in Merino ewes

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**Introduction** The plasma urea nitrogen (PUN) can be an indication of the quantity and degradability of the protein consumed. The high serum concentration of PUN may reduce fertility by local toxic effect on either the sperm, the ovum or the developing embryo (Jordan and Swanson, 1979). The objective of this study was to investigate the effect of high concentration of PUN following the increase of crude protein (CP) intake on ovarian response and fertilisation rate of superovulated ewes.

**Materials and Methods** Twenty-four Merino ewes, 3 to 4 years old with the average weight of 40.8±1.1 kg assigned randomly into two equal groups and kept in individual metabolism crates. Basal diet consisted of dry Pangula hay (CP 55g/kg DM) 850 g/head/d was fed to all ewes. Treatment group were supplemented with 450 g/head/d blue lupin (CP 320 g/kg DM) for 7 days prior to artificial insemination (AI). Superovulation was induced using a progesterone vaginal sponge (Chronogest 30, Intervet, Australia) for 12 d, 400 IU PMSG (Folligon, Intervet, Australia) and 12 mg FSH-P (Schering Corp. USA); FSH-P was injected inter muscularly (i.m) twice daily or 3 d starting 48 h before sponge removal. PMSG was injected i.m in a single dose at the same time as the first FSH-P dose. Embryos were collected surgically 7 days after laparoscopic AI with fresh semen. Daily blood sampling of five ewes in each group was carried out from Day –2 (Day 0=commencement of lupin feeding) to Day 10 of the experiment. The concentrations of PUN were determined using an enzymatic method (Enzymatic urea regent Trace, Scientific, Australia). Repeated measures analysis of Variance and the Wilcoxon test were used to analyse the changes in PUN and to compare superovulatory responses and embryo recovery rates, respectively.

**Results** The present study reveals a significant positive correlation between lupin-fed and control groups in terms of PUN. The mean concentrations of PUN (mmol/litre) increased significantly ($P<0.01$) after initiation of lupin supplementation to 14.56±0.5 mmol/litre on Day 2 of the experiment and remained at the same level ($P>0.05$) until Day 6 (Figure 1). On Day 8 of the experiment PUN levels declined significantly to5.6±0.8mmol/litre ($P<0.01$).

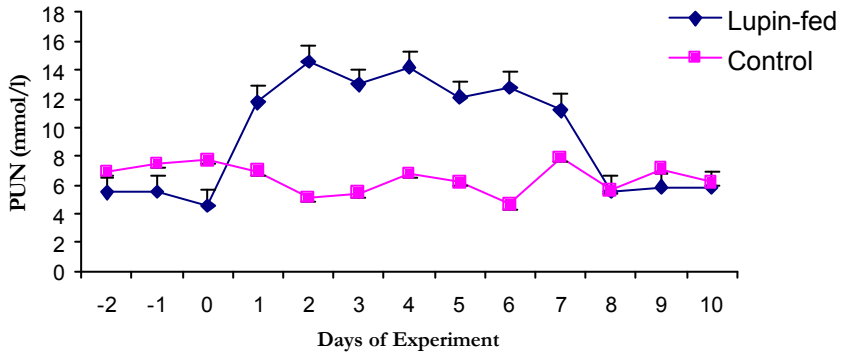


**Figure 1.** The plasma urea nitrogen (PUN, mmol/litre) in the control (n=5) and lupin-fed (n=5) ewes (Day 0=the first day of lupin supplementation).

In spite of increased PUN levels 6-7 days prior to AI, no significant differences were observed between lupin-fed and control groups in terms of number of corpora lutea (11±2.6 vs. 12±2.3), fertilised ova (5.8±1.6 vs. 5.9±1.2), unfertilised ova (0.8±0.6 vs. 1.6 ± 0.9) and transferable embryos (5.8±1.6 vs. 5.9±1.2). It might be due to a short period increase of PUN prior to ovulation followed by a dramatic decrease to a low level at the time of fertilisation.

**Conclusion** It is noticeable that the PUN changes in the lupin-fed animals still fall within the normal range (5.7 to 15.9 mmol/litre, McKenzie, 1992) and do not appear to have any detrimental effect on either superovulatory response or fertilisation rate. The results of this study indicated that the increase of PUN concentration after lupin feeding for 7days during superovulation has no detrimental effect on ovarian response and fertilisation rate in Merino ewes.

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# The effect of dilution rates and freezing methods on post-thawing motility of Baluchi ram spermatozoa

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**Introduction** For the effective use of Artificial insemination technique in sheep industry, investigation on the methods of ram semen dilution and freezing is necessary. Ahangari (1992) showed that various rates of dilution from 1:1 to 1:4 did not affect $p>0.05$ post thawing survival of Cambridge ram spermatozoa. Fiser et al (1987) achieved a 73% c.f. 67% pregnancy rate using thawed semen, previously frozen to -100 and -79 respectively. The objective of this study was to investigate the effect of two rates of dilution of semen and two methods of freezing on post-thawing motility of ram spermatozoa.

**Materials and methods** Three Baluchi rams were trained to ejaculate semen into an artificial vagina at Baluchi Sheep Breeding Station, Abbas-abbad, Mashhad, Iran. Baluchi is meat and carpet wool type sheep. They are raised extensively throughout the North to East of country. Semen was collected and assessed. Semen samples at a concentration of 4000 millions per ml with a good wave motion >3 were mixed. Pooled semen was diluted with tris buffer containing egg-yolk (15% v/v) at two rates of 1:1 or 1:2 (semen:diluent). Glycerol was added in two steps at +35 and +5 reaching to a final concentration of 4% (v/v). Diluted semen samples were cooled to +5 and then 0.5 ml straws were filled. Semen was frozen manually by placing straws in liquid nitrogen vapor using two methods. Straws were held at -120 in liquid nitrogen vapor for 6 minutes and then being plunged into a liquid nitrogen tank (fast freezing). Straws were gradually lowered in liquid nitrogen vapor at –70 to freeze within ten minutes and then being plunged into a liquid nitrogen tank (slow freezing). Post-thawing progress linear motility of ram semen samples were assessed and data was corrected using the following formula (Gill, 1978). Y=Arcsin (X/100) ^0.5, Y=corrected motility percentage and X=motility percentage. The experiment was planned on 2x2 factorial in a completely randomized design to examine two rates of dilution of semen and two methods of freezing with three replications. Mean comparison was carried out using Duncan multiple range tests at 0.01 probability level.

**Results** The corrected means of post-thawing motility of spermatozoa for treatments are shown in Table 1. The differences between rates of dilution, 1:2 or 1:1 on post-thawing motility of spermatozoa (27.56 Vs 12.9) was significant ($p<0.01$). This is in agreement with Mathur (1991) who suggested a similar rate of dilution for obtaining an optimum cryosurvival of ram spermatozoa. The differences between methods of fast freezing and slow freezing on post thawing motility of spermatozoa (23.46 Vs 16.32) was significant ($p<0.01$). This confirmed previous report of Ahangari (1992) that semen in straws, frozen to –100 and –120 before being plunged into liquid nitrogen survived better than semen frozen to –60 or –80.

**Table 1** The corrected mean (s.e.) of post-thawing motility of spermatozoa for each treatment ($p<0.01$)

| Methods of dilution and freezing | Corrected means (s.e.) of post-thawing motility |
|---|---|
| 1:1 and Fast | 13.97 (0.87)c |
| 1:1 and Slow | 9.90 (1.79)c |
| 1:2 and Fast | 33.17 (1.28)a |
| 1:2 and Slow | 22.65 (1.66)b |

**Conclusion** The dilution rate of 1:2 (semen:diluent) and using a manual method of fast freezing to -120 before being plunged into liquid nitrogen tank can be suggested for a long term storage of Baluchi ram semen in Iran.

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**Acknowledgements**

We would like to thank Natural Resources & Animal Research Center, Khorasan, Iran for their support and staffs of Baluchi Sheep Breeding Station for their help throughout this work.

# Effects of calcium salts of fatty acids on follicular characteristics and several blood parameters in two fat-tailed sheep breeds

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**Introduction** There is substantial evidence that the increased consumption of fat by dairy cattle can result in an increase in the number ovarian follicles, and the number and size of corpora lutea; it also stimulates post-partum ovarian activity and improve pregnancy rate. Increased dietary lipid also increases plasma cholesterol and progesterone, and the supply of lipoproteins which play significant roles in regulating ovarian steroidogenesis (Willimas, 1996). In contrast to cattle, there are few observations on the effects of dietary lipid on reproductive function in sheep. Intravascular infusion of lipid into ewes stimulated progesterone and prostaglandin synthesis (Burke *et al*., 1996), and dietary supplementation of calcium soaps of fatty acids enhanced luteal function (Kuran *et al*., 1999). Calcium soaps of fatty acids have been manufactured in Iran in recent years and sold under the trade name of Megalac. The aim of the present experiment was to study the effects of this protected fat on follicular number and luteal activity in two fat-tailed sheep breeds.

**Materials and methods** Oestrous cycles of twenty cyclic fertile ewes (4-5 years old) from each of two Iranian fat-tailed sheep breeds (Ghezel and Mehraban) were synchronised by two intra-muscular injections, 11 days apart, of cloprostenol. Within each breed, the ewes were randomly allotted to 4 groups. The control group was fed with a diet, balanced according to the NRC recommendations. The other groups received the same diet as well as a daily allowance of 40 g non-protected fat (NPF), 40 g protected fat (LPF), or 80 g (HPF) protected fat (Megalac). The ewes were gradually adapted over one week to the diets and were then fed with their complete rations for one cycle length, starting on the day of induced oestrus. Blood sample were taken on days 10, 12 and 14 of the cycle, and the sera were pooled for analysis of progesterone ($P_4$), cholesterol (CHOL), high density lipoproteins (HDL), low density lipoproteins (LDL), and triacylglycerols (TG). The ewes were slaughtered on their next oestrous period, and the size and number of follicles in ovaries were recorded. The follicles were classified into small (<4 mm), medium (4-7 mm), and large (>8 mm) groups. The data were analysed by using the GLM procedure of the Statistical Analysis System (SAS) and the means were compared by using the Tukey's test.

**Results** There were no significant effects of feeding fat on ovarian weights, cycle length, follicular numbers in each class, or the size of the largest follicle ($P>0.5$). However, there was an interaction between the diet and breed for the number of large follicles in the left ovary ($P<0.05$). In Ghezel breed, a greater number of large follicles was observed in the left ovaries of the LPF group, as compared with the control group. Serum concentrations of $P_4$, CHOL, TG, and HDL were greater for HPF ewes as compared with the control ewes ($P<0.05$). Serum concentration of LDL was not statistically different between the experimental groups (Table 1).

**Table 1** Mean concentrations of blood parameters in experimental ewes

| Diet | HPF | LPF | NPF | Control |
|---|---|---|---|---|
| $P_4$ (nmol $L^{-1}$) | $15.62^{a}$ | $11.76^{b}$ | $10.88^{b}$ | $8.44^{b}$ |
| CHOL (mg $dL^{-1}$) | $104.2^{a}$ | $82.8^{ab}$ | $87.5^{ab}$ | $66.5^{b}$ |
| TG (mg $dL^{-1}$) | $24.6^{a}$ | $24.3^{a}$ | $19.7^{b}$ | $18.5^{b}$ |
| HDL (mg $dL^{-1}$) | $49.2^{a}$ | $40.5^{b}$ | $46.9^{a}$ | $31.1^{c}$ |
| LDL (mg $dL^{-1}$) | $50.4^{a}$ | $38.7^{a}$ | $37.6^{a}$ | $33.2^{a}$ |

$^{a,b,c}$: Within each row, means with a common superscript (s) are not significantly different ($P>0.05$)

**Conclusions** Daily feeding of 80 g calcium salts of fatty acids (protected fat) for one cycle length did not change the number of follicles in different size groups, but significantly increased the serum concentration of $P_4$ between days 10 to 14 of the cycle which may be beneficial to early pregnancy maintenance in sheep.

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# The evaluation of *Acacia* and other tree pods for goats: influence of rumen fluid source and polyethylene glycol addition on *in vitro* gas production

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**Introduction** After prolonged exposure to tanniniferous diets, it has been reported that some rumen microorganisms acquire defensive mechanisms against tannins (Brooker *et al*., 2000) or produce tannin-degrading enzymes. Such rumen microorganisms are said to be "tannin resistant" as their fermentation activity is less inhibited by the presence of tannins in the host's diet. As acacia pods contain tannins their use as protein supplements for goats in the dry season may require that they be first detannified e.g. by using polyethylene glycol (PEG). However, goats with prior exposure to tanniniferous diets may have developed adaptive mechanisms to deal with tannins. This study, therefore, investigated the need for tannin inactivation in feeds given to 'adapted' animals by comparing the effect on the *in vitro* fermentation of tree pods incubated with and without PEG using rumen fluid from adapted and unadapted goats.

**Materials and Methods** Six rumen fistulated male Matebele goats were randomly allocated to either a commercial goat meal or a mixed tree pod supplement. The goats were penned individually and offered 200g supplements and 600g grass hay daily for 25 days. 'Adapted' rumen fluid was collected from the goats offered the mixed pods while the goat meal supplement group provided 'unadapted' rumen fluid for use in the Reading Pressure Technique (RPT) (Mauricio *et al*., 1999). Pods from *Acacia erioloba, A. erubiscens, A. nilotica, Dichrostachys cinerea* and *Piliostigma thonningii* trees, ground to pass through a 2mm sieve, were examined using a latin-square design (tree species x rumen fluid x PEG). Pod samples were incubated in triplicate (1g DM) for a period of 96 hours. Head-space gas pressure recordings were taken at 2, 4, 6, 8, 10, 12, 15, 19, 24, 30, 36, 48, 72 and 96 hours post incubation. Cumulative gas production data were analyzed for differences between factors and interactions using SAS GLM procedures.

**Table 1** Influence of rumen fluid and PEG (- / +) on 96 h cumulative gas release (ml)

| Species | Unadapted | | Adapted | | se mean |
|---|---|---|---|---|---|
| | - | + | - | + | |
| *A. erioloba* | 126.4 | 151.2 | 148.1 | 171.6 | 2.0 |
| *A. erubiscens* | 102.2 | 116.9 | 111.3 | 121.7 | 3.3 |
| *A. nilotica* | 98.5 | 132.2 | 146.9 | 170.3 | 2.5 |
| *D. cinerea* | 84.2 | 149.1 | 98.9 | 144.6 | 3.1 |
| *P. thonningii* | 107.9 | 160.5 | 126.9 | 166.2 | 2.7 |

**Results** Adapted rumen fluid consistently increased gas production over unadapted rumen fluid for both untreated and PEG treated pod samples. There was no significant interaction between rumen fluid type and PEG inclusion for all the species. Time to half asymptote was shorter in adapted rumen fluid. Combined fractional rate of gas production at 12h was consistently higher for adapted rumen fluid. Significant increases ($P < 0.05$) in gas production (46% for *D. cinerea*) were still obtained with PEG in adapted rumen fluid suggesting that tannin inactivation may still be needed even for adapted animals.

**Conclusions** Ruminal adaptive mechanisms may not be adequate to deal with the antinutritive effects of all tannins present thus tannin inactivation might still be necessary to ensure maximum utilization of tanniniferous protein supplements. Longer adaptation periods (>25days) may be required to allow the rumen microbial population to become fully adapted to dietary tannins. *In vivo* trials are required to further investigate these findings since other adaptive mechanisms may be complimenting ruminal responses.

**Acknowledgements** This publication is an output from a research project funded by the United Kingdom for International Development (DFID) for the benefit of developing countries. The views expressed are not necessarily those of DFID (R7351, Livestock Production Research Programme).

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# Tannin contents and *in vitro* digestibility of Brazilian browses

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**Introduction.** Brazil has arid regions where livestock production is limited by forage source. However, some native herbaceous legumes have a dry tolerance and had been used as animal feed. Some of those plants have anti nutritional compounds such as tannins that and can interfere on intake and digestibility of these plants. Tannins have a high affinity with proteins and could make these molecules unavailable for animal. Compounds as polyvinylpolypyrrolidone (PVPP) have been used on tannin studies, because it has more affinity with tannins than proteins. Based on that, it is possible to evaluate the nutritive potential of tanniniferous plants, using PVPP as an inhibitor of tannin effects. The aim of this work was to evaluate the effect of tannins on *in vitro* rumen fermentation.

**Material and methods.** Thirteen plant species commonly found in Brazil were used: 1 - *Medicago sativa*, 2 - *Anadenanthera macrocarpa*, 3 - *Myracrodruon urundeuva*, 4 - *Capparis flexuosa*, 5 - *Cajanus cajan*, 6 - *Gliricidia sepium*, 7 - *Sesbania sesban*, 8 - *Mimosa tenuiflora*, 9 and 10 - *Leucaena leucocephala* (sun-dried and fresh, respectively), 11 - *Sida cordifolia*, 12 - *Cordia leucocephala* and 13 - *Herissantia tiubae*. Crude protein (CP) and dry matter (DM) were determined according to A.O.A.C. (1995). Neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL) were determined according to Van Soest and Wine (1967). Total phenol (TP) was determined using Folin-Ciocalteu reagent, total tannins (TT) as the difference of phenolics before and after tannin removal using the PVPP as described by Makkar (2000). *In vitro* gas production assay was conducted according to Mauricio et al. (1999). About 1 g of each sample was weighed into each of six bottles, three with and three without 1 g of PVPP. The inocula were collected before the morning meal from mature Santa Inês whether sheep (rumen fistulated). Volume of gas produced in each bottle was recorded at 3, 6, 9, 12, 16, 24, 36, 48, 60, 72 and 96 h after inoculation time, using a pressure transducer. Relationships between data were tested where appropriate.

**Results.** Chemical compositions of tested plants are presented on Table 1. There were negative correlations between tannins content and gas production after 24, 48 and 96 h (G24, G48, G96, respectively) (Figure 1) either with or without PVPP. The Pearson's coefficients for samples without PVPP ($P<0.05$) were -0.68, -0.68 and -0.61, and with PVPP, -0.60, -0.59 and -0.53, respectively for TT and G24, G48 and G96. Graphically (Figure 1), it is possible to see that with higher tannin contents, PVPP effect was greater.

**Table 1**. Chemical composition ($g.kg^{-1}$ DM) of tested plants

| *Plant* | *NDF* | *ADF* | *ADL* | *CP* | *TP* | *TT* |
|---|---|---|---|---|---|---|
| *1* | 372 | 295 | 59 | 205 | 13 | 8 |
| *2* | 404 | 295 | 113 | 162 | 138 | 126 |
| *3* | 417 | 234 | 98 | 131 | 204 | 194 |
| *4* | 498 | 352 | 134 | 117 | 26 | 25 |
| *5* | 639 | 501 | 128 | 138 | 28 | 20 |
| *6* | 408 | 319 | 220 | 214 | 14 | 7 |
| *7* | 570 | 385 | 207 | 187 | 14 | 11 |
| *8* | 463 | 325 | 145 | 160 | 140 | 123 |
| *9* | 630 | 425 | 251 | 187 | 30 | 24 |
| *10* | 326 | 219 | 77 | 176 | 93 | 79 |
| *11* | 514 | 358 | 74 | 135 | 75 | 60 |
| *12* | 538 | 429 | 130 | 130 | 47 | 39 |
| *13* | 486 | 347 | 91 | 130 | 22 | 16 |
| *S.D.* | 96.3 | 78.5 | 59.6 | 32.0 | 61.5 | 58.4 |

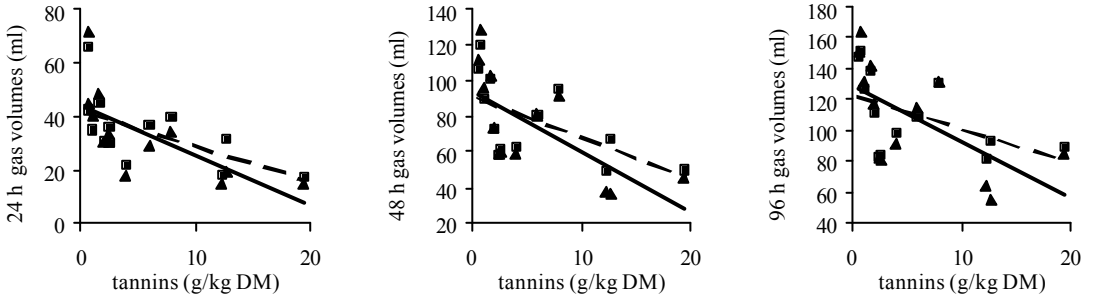


**Figure 1.** Relationships between tannin contents and gas production with (  ) or without (? ) PVPP. (Legend: continuous line - trend line of without PVPP data and dashed line - trend line of with PVPP data)

**Conclusions.** The study showed that tannins had negative effect on *in vitro* rumen fermentation and PVPP could show this effect.

**Acknowledgements.** This experiment is part of projects supported by FAPESP.

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# A comparison of solvents for extraction of condensed tannins in tree leaves.

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**Introduction.** The occurrence of tannins in plant leaves has a widely known ecological significance in terms of both nutritional constraint for animals and litter decay in the soil. However, there is no single protocol for extracting tannins for their subsequent quantification. The use of acetone has the advantage of inhibiting the polyphenol-protein interaction (Hagerman and Robbins, 1987), but becomes an disadvantage when assaying the protein precipitation capacity of the extract in biological assays (Hagerman and Butler, 1991). The objective of the present work was to compare tannin extracting agents and to assess the relationship arising between different solvents.

**Materials and methods.** Twenty two samples of forage tree leaves from central Mexico were analyzed for tannin contents by the vanillin reaction method (Price and Butler, 1978). Samples were air dry and milled with a hammer mill using a 1mm mesh. Tannins were extracted using methanol 100%, acetone 70%, ethanol 80% and methanol 50% with 2 hours at 80 $^{o}$C. Catequine was used as standard. Samples were analyzed by duplicate. Correlation and linear regression analysis was performed on the estimated amount of tannins with the four solvents.

**Results.** Total extracted tannins ranged from 0.0 to 32.6 (g/100 DM) with the different solvents used as extracting agents. Ethanol 80% gave consistently the lowest estimated and acetone 70% the highest (Table 1). Results were correlated ($P<0.01$) between most solvents, except ethanol and methanol-100% ($P>0.05$) (Table 2). With the exception of the acetone 70%-methanol 100% relationship, the remaining correlations were of low value for analytical purposes due to the resulting wide confidence interval.

**Table 1**. Condensed tannin contents on forage tree leaves as determined by the vanillin reaction in extracts with different solvents.

| Common / Scientific name | Aceto | Meth1 | Etha | Meth2 |
|---|---|---|---|---|
| **Dry season** | ------- g/100g DM * -------- | | | |
| Bellota /*Quercus rugosa* | 18.36 | 10.38 | 1.35 | 11.34 |
| Membrillo /*Amelanchier denticulata* | 32.67 | 10.78 | 0.85 | 23.37 |
| Azibuche /*Celtis sp.* | 1.36 | <1.0 | 0.18 | <1.0 |
| Tepozan /*Buddleia cordata* | 1.16 | <1.0 | 0.14 | <1.0 |
| Huizache /*Acacia farenesiana* | 24.53 | 3.74 | 0.94 | 12.11 |
| Mezquite /*Prosopis leviagata* | 0.74 | <1.0 | 0.13 | <1.0 |
| Pirul /*Schinus molle* | 19.79 | 4.49 | 1.10 | 10.79 |
| Madroño /*Arbutus xalapensis* | 36.80 | 22.52 | 1.01 | 8.54 |
| Tamarindo /*Dodonea viscosa* | 11.82 | 2.84 | 0.98 | 23.51 |
| **Rainy season** | | | | |
| Azibuche /*Celtis sp.* | 1.58 | <1.0 | 0.21 | <1.0 |
| Bellota /*Quercus rugosa* | 17.34 | 4.32 | 0.51 | 10.75 |
| Encino /*Quercus sp.* | 15.29 | 7.03 | 0.57 | 13.03 |
| Granjeno /*Celtis pallida* | 12.54 | 36.75 | 0.42 | 10.37 |
| Huizache /*Acacia farenesiana* | 17.35 | 5.43 | 0.50 | 8.06 |
| Espino /*Mimosa biuncifera* | 10.78 | 10.44 | 0.52 | 8.67 |
| Madroño /*Arbutus xalapensis* | 25.18 | 13.63 | 0.81 | 12.62 |
| Mezquite /*Prosopis leviagata* | 0.75 | 0.0 | 0.03 | <1.0 |
| Membrillo /*Amelanchier denticulata* | 24.59 | 14.29 | 0.57 | 15.63 |
| Palo dulce /*Eysenhardtia polystachya* | 1.82 | <1.0 | 0.06 | <1.0 |
| Pirul /*Schinus molle* | 11.17 | <1.0 | 0.26 | 5.90 |
| Tamarindo /*Dodonea viscosa* | 5.54 | <1.0 | 0.13 | 1.55 |
| Tepozan /*Buddleia cordata* | 1.83 | <1.0 | 0.12 | <1.0 |

*Catequine equivalent, Aceto:Acetone 70%, Meth1&2: Methanol-100 &50%, Etha: Ethanol 80%.

**Table 2.** Pearson correlation coefficient of condensed tannin content of forage tree leaves as extracted with different solvents.

| | Aceto. 70 | etha. 80 | meth 100 |
|---|---|---|---|
| Ethanol 80% | 0.790* | | |
| | 0.001** | | |
| Methanol-100% | 0.540 | 0.377 | |
| | 0.009 | 0.084 | |
| Methanol-50% | 0.966 | 0.752 | 0.613 |
| | 0.001 | 0.001 | 0.002 |

* Upper value in the cell: Correlation coefficient
** Lower value in the cell: P value

The best relationship obtained was; y = 0.116 (± 0.637 SE) + 0.620 (±0.037 SE), $P<0.001$, $R^2$: 0.929), where y: acetone- and b: methanol-50%-extracted tannins. Seasonal effects were not analyzed with the present dataset.

**Conclusion.** Acetone appeared to be the most efficient extracting agent for tannins in tree leaves. Methanol 50% was highly correlated with acetone, Thus, it can be used to predict the amount of tannins with acetone when the later extraction is not suitable for biological assays.

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# A comparison of solvents for extraction of polyphenolic compounds in tree leaves.

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**Introduction.** The occurrence of polyphenolics compounds in forage is of nutritional significance due to its protein binding capacity which can affect protein digestion (Woodward and Reed, 1989). They also have a role in controlling rates of decomposition and N mineralization of plant residues (Swift et al., 1979). The Prussian-blue assay (Price and Butler, 1977) is widely use for quantitative measurement of polyphenol content of plant materials due to the easiness it can be performed. Polyphenols are polar compound and literature report extractions with, methanol, ethanol or acetone as usual solvents. Although it is known that no single protocol is optimum for all samples. No comparison was found between solvents on a wide range of material to assess if results might be correlated. The objective of the present work was to compare the efficiency of four solvents for polyphenol extraction.

**Materials and methods.** Twenty two samples of forage tree leaves from central Mexico were analyzed for polyphenolic compounds by the Price and Butler (1977) method. Samples were air dry and milled with a hammer mill using a 1mm mesh. Polyhenols were extracted using methanol 100% (Price and Butler, 1977), acetone 70% (Hagerman and Robins, 1987), ethanol 80% (Bohn and Fales, 1991) and methanol 50% 2 hours at 80°C (Anderson and Ingram, 1989). Gallic acid was used as standard. Samples were analyzed by duplicate. Correlation analysis was performed on the estimated amount of polyphenols with the four solvents. The regression analysis were performed using the constant variance ratio approach as suggested by MacTaggart and Farwell (1992).

**Results.** Total extracted polyphenols ranged from 0.3 to 11.8 (g/100 DM) with the different solvents use as extracting agent. Ethanol 80% gave consistently the lowest estimated and acetone 70% the highest (Table 1). Results were correlated ($P<0.001$) between all solvents (Table 2). The best correlation for analytical purposes was that obtained from the acetone 70%-methanol 100% relationship. Seasonal effects were not analyzed.

**Table 1**. Polyphenolic contents on forage tree leaves as determined by the Prussian-blue reaction in extracts with different solvents*.

| Common / Scientific name | Aceto. | Meth1 | Etha | Meth2 |
|---|---|---|---|---|
| **Dry season** | --------- g / 100g DM -------- | | | |
| Bellota /*Quercus rugosa* | 5.23 | 2.57 | 1.73 | 3.94 |
| Membrillo/*Amelanchier denticulata* | 4.60 | 2.78 | 1.27 | 2.59 |
| Azibuche/*Celtis sp.* | 2.71 | 1.93 | 1.43 | 1.90 |
| Tepozan /*Buddleia cordata* | 2.05 | 1.43 | 0.63 | 1.20 |
| Huizache/*Acacia farenesiana* | 7.16 | 3.77 | 1.68 | 5.10 |
| Mezquite/*Prosopis leviagata* | 0.81 | 0.56 | 0.27 | 0.70 |
| Pirul/*Schinus molle* | 4.04 | 2.19 | 1.55 | 4.28 |
| Madroño/*Arbutus xalapensis* | 11.86 | 6.95 | 1.43 | 3.85 |
| Tamarindo/*Dodonea viscosa* | 3.51 | 2.36 | 0.99 | 6.81 |
| **Rainy season** | | | | |
| Azibuche/*Celtis sp.* | 2.96 | 2.31 | 1.43 | 1.75 |
| Bellota/*Quercus rugosa* | 4.92 | 3.28 | 2.14 | 4.32 |
| Encino/*Quercus sp.* | 7.59 | 3.98 | 3.00 | 7.24 |
| Granjeno/*Celtis pallida* | 2.77 | 1.46 | 0.85 | 3.86 |
| Huizache/*Acacia farenesiana* | 4.68 | 2.79 | 2.31 | 4.06 |
| Espino/*Mimosa biuncifera* | 10.11 | 6.32 | 4.80 | 7.74 |
| Madroño/*Arbutus xalapensis* | 8.81 | 5.14 | 4.45 | 7.48 |
| Mezquite/*Prosopis leviagata* | 0.57 | 0.67 | 0.36 | 0.70 |
| Membrillo/*Amelanchier denticulata* | 2.91 | 2.18 | 1.52 | 2.76 |
| Palo dulce/*Eysenhardtia polystachya* | 1.35 | 0.93 | 0.67 | 1.68 |
| Pirul/*Schinus molle* | 2.94 | 1.44 | 1.34 | 2.71 |
| Tamarindo/*Dodonea viscosa* | 2.21 | 1.46 | 1.14 | 2.10 |
| Tepozan/*Buddleia cordata* | 1.69 | 1.29 | 0.85 | 1.50 |

Aceto:Acetone 70%, Meth1&2: Methanol-100 &50%, Etha: Ethanol 80%. *Gallic acid equivalents.

**Table 2.** Pearson correlation coefficient of polyphenolic content of forage tree leaves as extracted with different solvents.

| | Aceto. 70 | Meth. 100 | Etha. 80 |
|---|---|---|---|
| Methanol 100% | 0.985 | | |
| Ethanol 80% | 0.748 | 0.759 | |
| Methanol 50% | 0.753 | 0.725 | 0.808 |

$P<0.001$ all correlations

The relationship between acetone and methanol-100% was: $y = 0.206 + 0.558$ (±0.100 95%CI for b), where y: acetone- and b: methanol-extracted polyphenols.

**Conclusion.** Acetone appeared to be the most efficient extracting agent for polyphenolic compounds. As methanol 100% was highly correlated with acetone, it can be used to predict total polyphenols with acetone when the later extraction is not suitable.

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# Rumen degradability for the assessment of tannin-rich forage from Brazil

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**Introduction.** Tannin can bind with feed nutrients and they became unavailable to ruminants. The aim of this work was to compare rumen degradability parameters with phenolic compounds of Brazilian ruminant feeds.

**Material and methods.** Thirteen samples of different species of plants were used: 1 - *Medicago sativa*, 2 - *Anadenanthera macrocarpa*, 3 – *Myracrodruon urundeuva*, 4 - *Capparis flexuosa*, 5 - *Cajanus cajan*, 6 - *Gliricidia sepium*, 7 - *Sesbania sesban*, 8 - *Mimosa tenuiflora*, 9 and 10 - *Leucaena leucocephala*, 11 - *Sida cordifolia*, 12 - *Cordia leucocephala* and 13 - *Herissantia tiubae*. Chemical composition was determined according to A.O.A.C. (1995). Neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL) were determined according to Van Soest and Wine (1967). Total phenol (TP) was determined using Folin-Ciocalteu reagents, total tannins (TT) as the difference of phenolics before and after tannin removal using the insoluble PVPP as described by Makkar (2000) and condensed tannins (CT) by Butanol-HCl method. In situ assays were conducted according to Ørskov and McDonald (1979) modified by McDonald (1981), using the following intervals: 4, 8, 16, 24, 48, 72 e 96 h. Each sample was weighted in triplicates and incubated in different animals (mature Santa Inês sheep). Data were compared by correlation using SAS system (SAS, 2000).

**Results.** Chemical compositions of tested plants are presented on Table 1. The mathematical model of Ørskov and McDonald (1979) fitted properly all tested feeds ($R^2$=0.90). Comparing values of potential (Pot) and effective degradabilities (Table 2), it is noticed that higher ED/Pot ratio corresponds to better potential use of feed by animal. On Table 3, correlations between TP or TT and rumen degradability parameters are presented. The greatest influence (P<0.05) of phenolic compounds (TP, TT or CT) was on degradability rate (c). Washing loss was not affected.

**Table 1**. Chemical composition (g.kg$^{-1}$ DM) of tested plants

| *Plant* | *NDF* | *ADF* | *ADL* | *CP* | *TP* | *TT* | CT |
|---|---|---|---|---|---|---|---|
| *1* | 372 | 295 | 59 | 205 | 13 | 8 | 0.3 |
| *2* | 404 | 295 | 113 | 162 | 138 | 126 | 9.0 |
| *3* | 417 | 234 | 98 | 131 | 204 | 194 | 43.5 |
| *4* | 498 | 352 | 134 | 117 | 26 | 25 | 1.4 |
| *5* | 639 | 501 | 128 | 138 | 28 | 20 | 6.2 |
| *6* | 408 | 319 | 220 | 214 | 14 | 7 | 0.3 |
| *7* | 570 | 385 | 207 | 187 | 14 | 11 | 1.9 |
| *8* | 463 | 325 | 145 | 160 | 140 | 123 | 69.2 |
| *9* | 630 | 425 | 251 | 187 | 30 | 24 | 10.0 |
| *10* | 326 | 219 | 77 | 176 | 93 | 79 | 65.4 |
| *11* | 514 | 358 | 74 | 135 | 75 | 60 | 97.2 |
| *12* | 538 | 429 | 130 | 130 | 47 | 39 | 0.2 |
| *13* | 486 | 347 | 91 | 130 | 22 | 16 | 0.3 |
| *S.D.* | 96.3 | 78.5 | 59.6 | 32.0 | 61.5 | 58.4 | 33.5 |

**Table 2.** Degradability rate (c, in h$^{-1}$), washing loss (A, in g.kg$^{-1}$ DM), insoluble fermentable fraction (B, in g.kg$^{-1}$ DM), potential degradability (Pot) and effective degradability (ED, in g.kg$^{-1}$ DM) at0.02 outflow rate

| *Plant* | *c* | *A* | *B* | *Pot* | *ED* | *ED/Pot* |
|---|---|---|---|---|---|---|
| *1* | 0.185 | 323 | 448 | 771 | 702 | 0.91 |
| *2* | 0.019 | 177 | 194 | 371 | 256 | 0.69 |
| *3* | 0.016 | 220 | 674 | 894 | 495 | 0.55 |
| *4* | 0.109 | 314 | 275 | 589 | 531 | 0.90 |
| *5* | 0.055 | 240 | 341 | 580 | 476 | 0.82 |
| *6* | 0.054 | 314 | 409 | 723 | 604 | 0.84 |
| *7* | 0.180 | 190 | 544 | 735 | 644 | 0.88 |
| *8* | 0.023 | 252 | 523 | 776 | 503 | 0.65 |
| *9* | 0.032 | 174 | 279 | 453 | 344 | 0.76 |
| *10* | 0.063 | 391 | 401 | 791 | 670 | 0.85 |
| *11* | 0.048 | 261 | 423 | 684 | 547 | 0.80 |
| *12* | 0.064 | 238 | 379 | 617 | 506 | 0.82 |
| *13* | 0.120 | 348 | 326 | 674 | 599 | 0.89 |
| *S.D.* | 0.064 | 64 | 133 | 148 | 126 | 0.11 |

**Table 3.** Relationship between phenolic compounds and rumen degradability parameters

| | *c* | *A* | *B* | *Pot* | *ED* |
|---|---|---|---|---|---|
| *TP* | -0.6083 *** | -0.2665 ns | 0.3827 * | 0.2292 ns | -0.3926 * |
| *TT* | -0.5923 *** | -0.2740 ns | 0.2290 ns | 0.2290 ns | -0.3949 * |
| *CT* | -0.4141 * | 0.0949 ns | 0.3725 * | 0.3725 * | 0.5001 ns |

**Conclusions.** Phenolic compounds as tannins have a negative influence on rumen degradability mainly on degradation rate of feed fermentable fraction.

**Acknowledgements.** This experiment is part of projects supported by FAPESP.

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# *In vitro* gas production of foliage and fruits of forage trees with and without added PEG

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**Introduction**. In Chiapas, México, natural vegetation is often used for grazing cattle. Local knowledge identify several plants and fruits as been consumed by cattle, But few information is available on their nutritive value. The objective of the present work was to assess the potential nutritive value and biological activity of tannins in 14 materials by means of the *in vitro* gas production technique.

**Materials and methods.** Nine foliage and 5 fruit samples from tropical trees where analyzed for their chemical composition (AOAC, 1980), total polyphenols (Price and Butler, 1977), and condensed tannin (CT) (Price et al., 1978). For the *in vitro* gas production (Theodorou *et al*., 1994) a N-rich media was employed. Rumen liquor from two cannulated cattle fed grass (*P. purpureum*) *ad lib.* plus 3 kgDM concentrate (180 gCP/kgDM) was collected before morning feeding. Liquor was filtered with cheese-cloth and mixed (1:1) with media; the remaining solid was blended with media (1:1) and filtered to obtain the final inoculum under constant flow of $CO_2$. Samples (0.5gDM) were incubated (4 replicates) with or without PEG-4000 (1g). Pressure and gas volume were measured at 3, 6, 9, 12, 15, 18, 21, 24, 30, 36, 48, 60, 72 and 96h. PEG effect on gas production was assessed with a "t" test.

**Table 1**. Chemical composition (g/kgDM) of forage trees foliage and fruits from Central Chiapas, Mexico

| Name | CP | OM | NDF | ADF | TP | CT |
|---|---|---|---|---|---|---|
| **Leaves** | | | | | | |
| *Leucaena leucocephala* | 201 | 898 | 275 | 191 | 3 | 13 |
| *Diphysa robinioides* | 187 | 882 | 317 | 232 | 6 | 15 |
| *Gliricidia sepium* | 238 | 894 | 385 | 247 | 3 | 0 |
| *Erythrina goldmanii* | 228 | 880 | 431 | 288 | 6 | 1.5 |
| *Genipa americana* | 94 | 915 | 377 | 309 | 9 | 2.7 |
| *Phitecollobium dulce* | 196 | 899 | 452 | 293 | 6 | 0 |
| *Guazuma ulmifolia* | 104 | 862 | 425 | 295 | 28 | 47 |
| *Acacia pennatula* | 125 | 929 | 590 | 358 | 28 | 40 |
| *Acacia milleriana* | 118 | 915 | 427 | 285 | 35 | 73 |
| **Fruits** | | | | | | |
| *Acacia milleriana* | 81 | 949 | 523 | 372 | 26 | 1.4 |
| *Enterolobium cyclocarpum* | 164 | 966 | 340 | 222 | 1.4 | 0.1 |
| *Leucaena leucocephala* | 186 | 942 | 519 | 370 | 13 | 1 |
| *Guazuma ulmifolia* | 58 | 947 | 461 | 354 | 6 | 2.8 |
| *Ficus glabrata* | 158 | 902 | 644 | 498 | 0.2 | 0.2 |

CP: crude protein; OM: organic matter; NDF, ADF: neutral and acid detergent fiber; TP: total polyphenols, CT: condensed tannins

**Results.** Most samples had moderate protein content, and similar fibre levels. CT were higher in *G. ulmifolia, A. milleriana*, and *A. pennatula* (Table 1). Both gas production and rate were increased by PEG in leaves but only rate on fruits (Table 2). PEG effect was different in foliage and fruits.

**Table 2.** *In vitro* gas production (ml/gDM) from forage tree foliage and fruits with and without PEG.

| Name | a | b | c | rsd | a | b | c | rsd |
|---|---|---|---|---|---|---|---|---|
| **Leaves** | | without PEG | | | | with PEG | | |
| *L. leucocephala* | -3±1.1 | 140±2.5 | 2.12±0.09 | 2.8 | -1±1.7 | 141±2.2 | 2.88±0.13 | 3.8 |
| *D. robinioides* | -16±2.5 | 188a±2.6 | 3.77±0.15 | 5.2 | -18±4.4 | 197b±4.4 | 3.96±0.25 | 8.9 |
| *G. sepium* | -12±1.7 | 175±1.8 | 3.83±0.11 | 3.5 | -10±4.1 | 174±4.1 | 3.92±0.26 | 8.2 |
| *E. goldmanii* | -16±2.1 | 174±2.2 | 3.71±0.14 | 4.4 | -17±3.2 | 176±3.2 | 3.72±0.20 | 6.5 |
| *G. americana* | -13±2.6 | 201±2.8 | 3.52±0.15 | 5.6 | -15±2.3 | 206±2.3 | 3.72±0.12 | 4.7 |
| *P. dulce* | -3±2.1 | 153±2.7 | 2.94±0.16 | 4.8 | -4±1.8 | 151±2.2 | 2.98±0.13 | 4.0 |
| *G. ulmifolia* | -6±1.9 | 168±5.8 | 1.80a±0.15 | 4.9 | -4±2.6 | 161±3.9 | 2.61b±0.19 | 6.0 |
| *A. pennatula* | -2±2.4 | 107a±5.3 | 2.12a±0.28 | 6.0 | -3±2.0 | 134b±2.4 | 3.12b±0.17 | 4.4 |
| *A. milleriana* | -3±1.5 | 134a±2.5 | 2.43a±0.13 | 3.5 | -7±1.9 | 150b±2.3 | 2.94b±0.14 | 4.2 |
| **Fruits** | | | | | | | | |
| *A. milleriana* | 2±1.9 | 119±2.9 | 2.59a±0.19 | 4.4 | 0.2±1.4 | 122±1.5 | 3.47b±0.13 | 3.0 |
| *E. cyclocarpum* | -21±3.8 | 215±3.7 | 4.97±0.21 | 7.0 | -25±5.1 | 221±4.8 | 5.39±0.27 | 8.9 |
| *L. leucocephala* | -8±1.7 | 172±2.2 | 2.85±0.11 | 3.8 | -8±1.6 | 177±1.9 | 3.03±0.10 | 3.5 |
| *G. ulmifolia* | -1±3.1 | 167±3.1 | 4.03a±0.21 | 6.2 | -5±2.2 | 157±2.1 | 5.57b±0.17 | 3.8 |
| *F. glabrata* | -1±1.5 | 72±3.47 | 2.08±0.26 | 3.8 | -3±1.1 | 73±2.4 | 2.17±0.19 | 2.8 |

a,b and c: fitted parameters of the equation gas=a+b(1-$e^{-ct}$), rsd. residual square deviation
values of a parameter in the same line with different letter differ at P<0.05

**Conclusion.** *In vitro* gas production and chemical composition South México forrage trees indicate their potential nutritive value as ruminant feed. High tannin activity was only detected on 3 species.

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# The effects of quebracho tannins on rumen degradation and post-rumen digestion of pea and lupin seeds

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**Introduction** The use of alternative protein sources in ruminant diets is of interest because of restrictions on the use of animal proteins, loss of nitrogen (N) compounds to the environment and efforts to reduce costs of systems. A problem of some protein-rich-ingredients is that they degrade rapidly in the rumen. The use of tannins has been proposed as a means of reducing the extensive dietary protein degradation in the rumen. However, condensed tannins have been reported to exert detrimental effects on gut microflora and on the gastrointestinal tract, and they can affect post-rumen digestion. The two experiments reported here aimed to study the influence of quebracho tannins (QTs) on ruminal degradation of peas (*Pisum sativum*) and sweet lupins (*Lupinus luteus*), and to investigate the effect of QTs on post-rumen digestibility of these feeds using a poultry model.

**Materials and Methods** Peas (DM= 940 g/kg; CP= 229 g/kg DM; ash= 31 g/kg DM) and lupins (DM= 934 g/kg; CP= 460 g/kg DM; ash= 43 g/kg DM) were ground to a particle size of 2 mm. QTs were added to ground material at two levels of inclusion. The following treatments of peas (P) and lupins (L) were obtained: P0, PQT50 (50 g of QT/kg CP), PQT150 (150 g of QT/kg CP), and L0, LQT50 (50 g of QT/kg CP) and LQT150 (150 g of QT/kg CP).
In experiment 1, 5 g of treated and untreated peas and lupins were incubated in duplicate in nylon bags (pore size of 40 um) in the rumen of each of three sheep for 3, 6, 9, 12, 16, 24, 48, and 72 h. After incubation the bags were washed with cold water in a commercial washing machine. They were then dried in a forced-draught oven at 45ºC and weighed to determine DM disappearances. Differences between treatments observed in DM disappearance of peas (P0, PQT50 and PQT150) and lupins (L0, LQT50 LQT150).were subjected to a repeated measure arrangement (GLM).
The observed DM disappearances were fitted to the exponential equation described by Ørskov and McDonald (1979)[$d = a + b(1\text{-}e^{(-ct)}$], where $d$ represents the loss from the bag after $t$ hours, $a$ the rapidly degradable fraction, $b$ the slowly degradable fraction and $c$ the rate of degradation of fraction $b$. Differences between treatments of the *in situ* kinetic characteristics were analysed using the Kruskal-Wallis test.
In experiment 2, broilers (approx 3 kg) were used in a precision feeding study (Sibbald, 1976) of 8 replicates per feed treatment. Each bird received about 20 g of PQT50, LQT50, P0 and L0. Excreta voided during the 48 h following feeding were quantitatively and individually collected, frozen (-20ºC), freeze dried then weighed and finely ground (1 mm). The endogenous losses were estimated from the (control) birds fed glucose. Differences between treatments measured in true DM digestibility (TDMD) and coefficient of nitrogen retention (CNR) were analysed using a t-test.
All data (Experiment 1 and 2) were analysed using the procedures of the SPSS statistical package.

**Results** In experiment 1, no differences (P>0.05) were observed in the DM disappearance between the 2 levels of QTs for peas and lupins throughout the incubation period. Although, QTs decreased DM disappearance of lupins until 9 h of incubation compared with the control (L0), interestingly the same effect was not observed in peas. Tannin-treated peas had higher DM disappearance than the untreated peas for all incubation time. Ruminal degradation of the treatments for peas ($R^2 = 0.92 \pm 2.747$) and lupins ($R^2 = 0.98 \pm 1.738$) was described by the exponential equation defined by Ørskov and McDonald (1979). The data clearly shows that the coefficient of determination for peas was considerably lower than that observed in lupins. In experiment 2 (see Table 1), no effect (P>0.05) of tannins was found in true dry matter digestibility (TDMD) coefficient of lupins and peas measured in poultry. Tannin treatment decreased (P<0.05) the coefficient of nitrogen retention (CNR) compared to untreated lupins. There was a tendency for tannins to reduce nitrogen retention with peas but the variation was very large for the tannin treated peas compared to the untreated peas.

Table 1 Mean values and standard errors of TDMD and CNR for all treatment feeds (Experiment 2).

| Feed Treatments | TDMD | CNR |
|---|---|---|
| P0 | 0.75 ± 0.03 | 0.62 ± 0.12 |
| PQT50 | 0.77 ± 0.05 | 0.09 ± 0.40 |
| L0 | 0.70 ± 0.04 | 0.78 ± 0.08 |
| LQT50 | 0.55 ± 0.07 | 0.33 ± 0.12 |

**Conclusions** Tannins depress ruminal degradation of feeds and this was observed with lupins but not for peas. Protein rich seeds (such as peas and lupins) contain important amounts of starch and non-starch polysaccharides, and their properties as tannin-binding agents should be investigated. The ingestion of tannins has been demonstrated to increase endogenous losses and variability in nitrogen retention due to lesions of the gut mucosa. This may account for some of the results observed, particularly, in tannin-treated peas.

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# Intake, digestibility and microbial-N synthesis in Creole goats fed grass/forage tree silage

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**Introduction.** Forage trees are commonly used in the tropics as supplementary feed for ruminants**.** However, during the dry season where grass is of poor quality, many trees also shed their leaves and are no longer available. Adequate strategies are to be evaluated to allow forage trees to be introduced into feeding systems as good quality supplements along the year. Silage might be an adequate technology if the resultant product allow similar animal performance as those achieved using commercial concentrate as supplement, but few studies have been conducted with forage tree silages. The objective of the present experiment was to evaluate intake, digestibility and microbial-N synthesis of diets supplemented with grains or forage tree silage.

**Material and methods**. *Albizia lebbeck* and *Piscidia piscipula* silage was prepared using 230kg tree, 60kg *Pennisetum purpureum* grass and 10 kg sugarcane molasses (fresh base). After 90-d silages were opened and six lactating goats (38 ± 3.5) were used in a randomized block design (period as blocking factor). Diets (four replicates per treatment) were a) grass + 135 gDM concentrate ( sorghum/soybean, 18% CP) as control diet, b) grass and *A. lebbeck* silage and c) grass + *P. piscipula* silage experimental diets were offered *ad libitum* in 40% grass-60% silage ratios (fresh base). Goats were kept in metabolic crates where total faeces and urine were collected over 5-d periods, after 10-d of adaptation to each diet. Every morning feed intake was measured by difference between offered and refused feed. Faeces and urine were weight a kept frozen for analyzes. Twenty ml sulphuric acid 25% v/v were added to urine before frozen. Microbial-N synthesis was estimated using the urinary purine derivative technique (Chen *et al.*, 1990). Samples of feed and faeces were analyzed for chemical composition (AOAC, 1980), silage samples were additionally analyzed for pH, polyphenols and condensed tannins (Price and Butler 1977, Price *et al,* 1978).

**Results.** Forage tree silage presented good qualities at opening (90-d) suggesting that forage trees can be preserve as silages (Table 1). No differences were found on intake and digestibility of diets (Table 2). However, animals with *A. lebbeck* silage as supplement had a higher N-balance than *P. piscipula* and control diets (P<0.001), partly due to the higher DMI and the CP content of the grass + albizia silage diet (73, 93 and 79 gCP/kgDM for grass, albizia and piscidia diets respectively). No difference was found on urinary purine derivatives excretion and estimated microbial-N synthesis between diets, suggesting that *A. lebbeck* higher N-balance could be arising from protein resisting microbial breakdown probably due to the presence of tannin-protein complexes on leaves, protein being further available at intestinal level (Diaz-Hernandez *et al.*, 1997). Silage process seems to preserve this forage characteristic.

**Table 1.** Chemical composition of diets (g/kg)

| | Grass | A. lebbeck | P. piscipula |
|---|---|---|---|
| DM | 330 | 340 | 310 |
| OM | 911 | 918 | 900 |
| CP | 63 | 128 | 95 |
| ADF | 395 | 463 | 449 |
| NDF | 660 | 674 | 564 |
| pH | -- | 4.88 | 4.57 |
| TP | -- | 0.09 | 0.08 |
| CT | -- | <1 | <1 |

TP: total poliphenols, CT: condensed tannins

**Table 2.** Intake, digestibility and N balance of experimental diets

| | Control | A. lebbeck | P. piscipula | SEM |
|---|---|---|---|---|
| DMI (g/d) | 913 | 1105 | 921 | 97.5 |
| DMD | 0.528 | 0.589 | 0.540 | 0.0252 |
| OMD | 0.555 | 0.606 | 0.556 | 0.0258 |
| ADFD | 0.520 | 0.582 | 0.530 | 0.0354 |
| NDFD | 0.525 | 0.613 | 0.556 | 0.0363 |
| DDMI (g/d) | 482 | 651 | 0.494 | 57.2 |
| N-balance (g/d) | 33.6a | 66.8b | 33.2a | 6.42 |

**Table 3.** Urinary purine derivatives (mg/d) excretion and microbial-N synthesis of experimental diets.

| | Control | *A. lebbeck* | *P. piscipula* | SEM |
|---|---|---|---|---|
| Xhantin-Hipoxhantin | 83.5 | 56.8 | 55.7 | 11.23 |
| Uric acid | 265.6 | 242.9 | 213.3 | 29.37 |
| Allantoin | 724.1 | 737.4 | 493.4 | 116.73 |
| Total PD (mmol/$LW^{0.75}$) | 0.42 | 0.43 | 0.33 | 0.050 |
| Microbial N (g/d) | 5.43 | 5.20 | 3.44 | 0.860 |

**Conclusion.** Silage could be used as means of preserving forage tree for the dry season especially for those species that shed leaves. Forage tree silage may be used as replacement of conventional concentrates based on cereals.

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# Milk yield in Creole goats fed grass/forage tree silage

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**Introduction.** In tropical smallholding, goats are a common source of milk either for sale of self-consumption. Concentrates are commonly use to support milk production during the dry season where many trees shed their leaves and are no longer available for feeding. Silage making might be an adequate technology for using forage trees as quality supplements along the year. Few studies have been conducted with forage tree silages to evaluate the resultant animal performance especially regarding milk production. The objective of the present experiment was to evaluate milk production and quality of milk of goats fed grass and supplemented with grains or forage tree silage.

**Material and methods**. *Albizia lebbeck* and *Piscidia piscipula* silage was prepared using 230kg tree, 60kg *Pennisetum purpureum* grass and 10 kg sugarcane molasses (fresh base). After 90-d silages were opened and six lactating goats (38 ± 3.5 Kg LW) were used in a randomized block design (period as blocking factor). Diets (four replicates per treatment) were a) grass + 135g DM concentrate ( sorghum/soybean, 18% CP) as control diet, b) grass and *A. lebbeck* silage and c) grass + *P. piscipula* silage experimental diets were offered *ad libitum* in 40% grass-60% silage ratios (fresh base). Goats were kept in individual crates and hand-milked every morning. Experimental period for collecting data was 5-d after 10-d of adaptation to each diet. Every morning feed intake was measured by difference between offered and refused feed. Samples of milk were analyzed for fat (Gerber method, ILCA, 1988), protein, total solids and urea (AOAC, 1980). Silage samples were additionally analyzed for pH, total polyphenols and condensed tannins (Price and Butler 1977, Price *et al,* 1978).

**Results.** The amount of concentrate used was similar to those employed by local farmers as supplement. It use along with the grass provided a diet with a nutritive value similar to the grass + *P. piscipula* diet, and both of them were of a slightly lower nutritive value than the grass + *A. lebbeck* diet in terms of chemical composition (Table 1). No differences were found on intake, milk yield, milk composition and yield of milk constituents between diets (Table 2).

**Table 1.** Chemical composition (g/kg except pH) of diets fed to goats

| | Grass + concentrate | Grass + *A. lebbeck* | Grass + *P. piscipula* |
|---|---|---|---|
| DM | 364 | 340 | 310 |
| OM | 911 | 921 | 900 |
| CP | 73 | 99 | 79 |
| ADF | 325 | 448 | 619 |
| NDF | 545 | 643 | 650 |
| pH | -- | 4.88 | 4.57 |
| TP | -- | 0.09 | 0.08 |
| CT | -- | <1 | <1 |

TP: total polyphenols, CT: condensed tannins, ADF, NDF: acid and neutral detergent fibre.
pH, TP & CT: measured on silage alone

**Table 2.** Intake and milk production and composition of goat fed grass and supplemented with forage tree silage

| | Control | *A. lebbeck* | *P. piscipula* | SEM |
|---|---|---|---|---|
| DMI (g/d) | 913 | 1105 | 921 | 97.5 |
| Milk yield (g/d) | 211 | 273 | 197 | 46.3 |
| Fat (g/kg) | 27.7 | 18.3 | 35.2 | 5.35 |
| Protein (g/kg) | 40.8 | 34.0 | 33.7 | 4.88 |
| Total solids (g/kg) | 140.6 | 126.5 | 136.1 | 9.69 |
| Urea (mg/kg) | 26.8 | 29.0 | 24.4 | 1.47 |
| Fat yield (g/d) | 5.9 | 4.8 | 6.1 | 1.02 |
| Protein yield (g/d) | 7.8 | 9.2 | 6.4 | 1.12 |
| Milk solids yield (g/d) | 28.0 | 34.5 | 26.0 | 4.54 |

DMI: Dry matter intake

A higher milk production with a reduced fat content appeared to result with the use of the A. lebbeck silage, but, due to the large variation found it was not a significant effect (P>0.1). Further studies will be needed in order to clarify this effects.

**Conclusion.** Animals supplemented with forage tree silage achieved similar production levels to those using conventional concentrates (based on cereals) as supplements.

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# Relationship between *in vivo*, *in situ* and *in vitro* techniques for evaluation of tropical forages in Brazil

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**Introduction.** *In vivo* experiments are the preferred method for ruminant feed evaluation, but they are very expensive, laborious and time-consuming. *In situ* and *in vitro* techniques are commonly used as a routine all over the world as a predictor of *in vivo* results. *In situ* assays have been the basis of many feed evaluation systems due to its ease of use and low cost. *In vitro* techniques, such as gas production, give an opportunity to get similar information plus a better description of fermentative kinetics. The aim of this work was to compare data obtained from *in vivo*, *in vitro* and *in situ* assays for the evaluation of three tropical forages used in ruminant nutrition in Brazil.

**Material and methods.** Three forage hays (*Brachiaria decumbens*, Tifton - *Cynodon* X *Cynodon* - and tropical Lucerne - *Medicago sativa*) were evaluated by *in vivo*, *in situ* and *in vitro* techniques. The animals used in all experiments were Santa Inês wether sheep with permanent rumen fistulas. Diets were based exclusively on each corresponding hay and the only supplementation was a commercial mineral mixture. The statistical design was a double 3x3 Latin square, with 3 treatments, 3 periods and 6 animals. Each experimental period for *in vivo* assays was 28 days long divided in 9 days for change-over and adaptation to diets, 10 days for voluntary intake measurements, 4 days for adaptation to metabolic cages and 5 days for total collection of faeces for apparent digestibility measurements. *In situ* assays were conducted according to Ørskov and McDonald (1979) modified by McDonald (1981) during the last 4 days of the change-over and adaptation stage of the *in vivo* assay. The *in vitro* gas production assay was conducted according to Mauricio *et al*. (1999), with degradability measured at pre-determined intervals. The inocula were collected directly from the rumen, at the first day of voluntary intake measurements of *in vivo* assay. *In vitro* and *in situ* degradabilities were fitted to the $p=a+b(1-e^{-ct})$ model. Data were compared using SAS for Windows software (SAS, 2000), using a correlation procedure.

**Results.** There were no significant effects of period or animal. The only observed effects ($P<0.01$) were due to treatment (diets). The results of the correlation approach are presented in Table 1. All data presented on Table 1 are in a straight relationship. The lowest Pearson's coefficients (r) were observed between DM intake and apparent digestibility. Comparing techniques, the highest r-value was detected between *in situ* and *in vitro* techniques and both are associated with *in vivo* results in a similar way, which becomes possible to predict *in vivo* digestibility.

**Table 1**. *Relationship between* in vivo, in situ *and* in vitro *assays*

| *Variables* | $ED_{vitro}$ | | *DMI* | | *DMD* | | *OMD* | |
|---|---|---|---|---|---|---|---|---|
| | *r* | *P* | *r* | *P* | *r* | *P* | *r* | *P* |
| $ED_{situ}$ | 0.959 | <0.0001 | 0.865 | <0.0001 | 0.782 | 0.0002 | 0.731 | 0.0009 |
| $ED_{vitro}$ | | | 0.810 | <0.001 | 0.812 | <0.0001 | 0.777 | 0.0001 |
| *DMI* | | | | | 0.642 | 0.0040 | 0.580 | 0.0117 |
| *DMD* | | | | | | | 0.991 | <0.0001 |

$ED_{situ}$ – *in situ* dry matter effective degradability, $ED_{vitro}$ – *in vitro* dry matter effective degradability, *DMI* – dry matter voluntary intake, *DMD* – *in vivo* dry matter apparent digestibility, *OMD* - *in vivo* organic matter apparent digestibility, *r* – Pearson's correlation coefficient, *P* – probability.

**Conclusion.** The i*n situ* and *in vitro* techniques could estimate *in vivo* results, regarding to Brazilian ruminant feed evaluation.

**Acknowledgements.** This experiment is part of a project supported by FAPESP.

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# Voluntary intake and apparent digestibility of tropical forages fed to sheep in Brazil

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**Introduction.** In Brazil ruminant production is based mainly on extensive systems in which forages are the most important, if not the only, source of nutrients. The quality of tropical forages declines seriously with advancing maturity and in extensive systems large amounts of forage commonly remain unused after grazing during the wet season (spring/summer). This unused forage could be harvested and sun-dried. During the dry season, there is a lack of feed on pastures and the hay produced from unused forages could provide a cheap alternative to concentrate supplements. The objective of this experiment was to evaluate three commercially available tropical forages as sheep feed in the Piracicaba river region of Sao Paulo State in Brazil.

**Material and methods.** As treatments, three commercially available sun-dried hays (BRA = *Brachiaria decumbens*; TIF = Tifton - *Cynodon* X *Cynodon;* and LUC = tropical Lucerne - *Medicago sativa*) were fed to Santa Inês wether sheep (LW=39.9±5.7). Diets were based on the experimental hays alone and the only supplementation was a commercial mineral mixture. The statistical design was a double 3x3 Latin square with 3 treatments, 3 periods and 6 animals. Each experimental period was 28 days long with 9 days for change-over and adaptation, 10 days for voluntary intake measurements, 4 days for adaptation to metabolic cages and 5 days for apparent digestibility measurements by total collection of faeces. Diets were fed twice a day (8:30 and 16:30h) and animals had free access to water. To ensure *ad libitum* intake, during the first 19 days (change-over and voluntary intake stages), diets were offered at 10-20% above measured intake. After this period (during the last 9 days), to minimise selection by the sheep, diets were fed at 90% of measured voluntary intake. During the collection period, samples of offered and refused meals and faeces were collected for calculating dry matter or organic matter apparent digestibility. Data were compared using SAS for Windows software (SAS, 2000) using a least square means approach.

**Results.** The chemical composition of the diets is presented on Table 1. Compared with LUC, values for the grasses for neutral detergent fibre (NDF) were much higher and those for crude protein (CP) much lower. NDF values above 700 $g.kg^{-1}$ DM are very common in tropical grasses as cell walls are very thick as a heat protection mechanism. Treatments had significant effects ($P<0.05$) on feed intake and digestibility (Table 2). Dry matter voluntary intake of diet LUC was greater than BRA and TIF. There was a similar pattern for dry and organic matter apparent digestibilities, which could be explained by the higher fibre contents of tropical grasses than LUC (a legume). Voluntary intake and apparent digestibility of BRA and TIF did not differ and they can be considered very low even for tropical grasses. Nitrogen content of BRA and TIF was very low and a large part of it is strongly linked to fibre and is almost insoluble (Table 1).

**Table 1.** *Chemical composition* ($g.kg^{-1}$ DM) of the forages*

| *Diets* | *OM* | *NDF* | *ADF* | *ADL* | *ADIN* | *Total N* | *CP* |
|---|---|---|---|---|---|---|---|
| *BRA* | 927 | 759 | 444 | 43 | 2.7 | 4.8 | 30 |
| *TIF* | 912 | 775 | 442 | 62 | 4.6 | 10.8 | 67 |
| *LUC* | 911 | 538 | 423 | 92 | 8.1 | 29.0 | 181 |

* *OM* – organic matter; *NDF* – neutral detergent fibre; *ADF* – acid detergent fibre; *ADL* – acid detergent lignin; *ADIN* – acid detergent insoluble nitrogen; *CP* – crude protein (6.25 x total nitrogen).

**Table 2.** *Voluntary intake and apparent digestibility of diets*

| *Diets* | *Voluntary intake* ($g.kg^{-1}$ *LW)* | *Apparent digestibility* | |
|---|---|---|---|
| | | *Dry matter* | *Organic matter* |
| *BRA* | $19.16^{b}$ | $0.470^{b}$ | $0.500^{b}$ |
| *TIF* | $22.96^{b}$ | $0.446^{b}$ | $0.463^{b}$ |
| *LUC* | $35.54^{a}$ | $0.552^{a}$ | $0.569^{a}$ |
| *s.e.d.* | 1.374 | 0.0151 | 0.0160 |
| *P value* | < 0.001 | < 0.01 | < 0.05 |

$^{a,b,c}$ means within columns with different superscripts are significantly different (see *P* value on table)

**Conclusion.** The commercially available tropical grasses have lower nutritional values than the tropical Lucerne and are unlikely to meet the requirements of sheep for growth.

**Acknowledgements.** This experiment is part of a project supported by FAPESP.

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# The effect of wilting or soaking on the nutritive value of two invasive weed species in Nepal

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**Introduction** Goats are an important component of the livelihoods of resource poor livestock keepers (RPLK) in Nepal. A major constraint is the poor health (and low economic value) of goats in the early part of the wet season, and this is partly brought about by the shortage of available forage in the dry season. Two invasive weeds (*Eupatorium adenophorum*, EA, and *Chromolaena odorata*, CO) now grow throughout Nepal. The plants grow year round, and so could be used as a source of forage, but their voluntary intake and perceived nutritive value by goats is low. If an appropriate means of treating EA and CO could be developed, their nutritive value may increase. EA and CO could then be included in the forage harvested for goats. The objective of this experiment was therefore to determine the effect on the nutritive value of EA and CO of either wilting or soaking these plants.

**Materials and methods** Samples of EA and CO were harvested in November, 2000 at the beginning of the dry season. Samples were collected from the forests used by the communities with whom the project is working. CO was taken from the district of Dhanusha, in the plains, whereas EA was collected from Makawanpur, in the mid hills. Each sample was split into three, and either left untreated (U), wilted for 2 h (W) or soaked in water for 2 h (S). Samples were then oven dried ($60^0$C), before being ground (1 mm screen). They were then analysed for organic matter (OM), crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF). Samples (1 g) were also incubated in triplicate on two occasions for 72 h with buffered rumen fluid taken from three sheep to estimate the rate and extent of gas production (GP). At the end of the incubation period, the samples were filtered through glass fibre paper (Whatman GF/A) to estimate degradability. The GP profiles obtained were fitted to a modified Michaelis-Menten model and from this, estimates of the total volume of gas produced ($a$), the time to half total gas volume ($k$), the maximum rate of gas production ($R_{M\ Gas}$) and the time to $R_{M\ Gas}$ ($t_{RM\ Gas}$) were calculated. Analysis of variance was used to determine the effects of treatment and species on the estimates of degradability and the parameters of the GP profiles obtained.

**Results** CP, NDF and ADF content, and the ADF:NDF ratio was higher for CO than EA (Table 1). The ADF content, and ADF:NDF ratio was reduced by both wilting and soaking. Total gas production and degradability of CO was lower than EA (Table 2). Soaking increased the degradability of EA, but had no effect on CO, while wilting increased the degradability of both species. Wilting also increased $R_{M\ Gas}$, particularly for CO.

**Table 1** Chemical analysis of *E. adenophorum* and *C. odorata*

| | Chemical composition, g/kg DM | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | OM | | CP | | NDF | | ADF | | ADF/NDF | |
| | EA | CO | EA | CO | EA | CO | EA | CO | EA | CO |
| U | 929 | 979 | 111 | 146 | 492 | 533 | 449 | 506 | 0.912 | 0.949 |
| W | 924 | 918 | 114 | 161 | 485 | 546 | 413 | 505 | 0.852 | 0.925 |
| S | 926 | 929 | 118 | 133 | 494 | 525 | 400 | 430 | 0.810 | 0.819 |

**Table 2** Effect of treatment on the degradability and GP profile of EA and CO

| | *E. adenophorum* | | | *C.odorata* | | | SEM | Significance of contrast | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | U | W | S | U | W | S | | Sp[1] | Tr[1] | SpxTr[1] |
| Degradability | 0.456 | 0.488 | 0.484 | 0.362 | 0.445 | 0.373 | 0.0392 | *** | *** | *** |
| $a$ (ml) | 161 | 182 | 174 | 133 | 156 | 135 | 11.4 | * | ns | ns |
| $k$ (h) | 8.8 | 8.8 | 8.7 | 10.1 | 7.8 | 9.8 | 0.71 | ns | ns | ns |
| $R_{M\ Gas}$ (ml/h) | 11.4 | 13.2 | 12.6 | 9.9 | 16.4 | 9.8 | 0.73 | ns | ** | * |
| $t_{RM\ Gas}$ (h) | 2.5 | 2.1 | 3.3 | 0.8 | 0.3 | 1.0 | 0.65 | * | ns | ns |

[1]Sp: species, Tr: treatment, SpxTr: species x treatment interaction ns not significant, * P<0.05, *** P<0.001

**Conclusions** EA appears to have a higher nutritive value than CO. Soaking had only a limited effect on EA, and none on CO, whereas wilting increased the nutritive value of both species. Wilting is therefore the more effective treatment. If acceptable to farmers, this intervention would increase the effective availability of forage in the dry season, and thereby help to improve the livelihoods of RPLK in Nepal.

**Acknowledgements** This document is an output from a project funded through the Livestock Production Programme of the UK Department for International Development (DFID) for the benefit of developing countries. The views expressed are not necessarily those of DFID. Project R7632, Livestock Production Programme.

# Degradability chracteristics of dry matter and crude protein of Iranian forages in ruminant

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**Introduction** Values for crude protein (CP) and ruminally undegradable CP content are now required in feed evaluation systems currently used in North America (NRC,1989) and other organizations (Ørskov,1992).The objectives of the present study were to assess in situ ruminal degradability ,fractional rates of digestion and the effective degradability of the dry matter (DM) and crude protein (CP) of alfalfa and red clover hays, selected randomly from dairy farms of Tabriz region in Iran on the male sheep rumen.

**Materials and methods** 10 alfalfa (Medicago Sativa ) and red clover hays were evaluated using the in situ rumen technique.Hay samples were collected from individual representatives of the Feed Manufacturing Industry field staff by subsampling at least five to seven bales using a bale core sampling device prior to pooling,thorough mixing and obtaining a composite sample.The forage samples were then ground through a 2 mm screen prior to incubation. Four male sheep were fitted with a rumen fistula. They were fed a diet consisting of an hay (alfalfa or red clover) (600 $gkg^{-1}$) and concentrate (400 $gkg^{-1}$) .Naylon bags were placed in the rumen 96,72,48,36,24,16,4 or 0 h befor removal at a common time in order to minimize the variation in time that the bags were exposed to air after incubation .The following equation developed by φrskov and McDonald (1979) Was used $P=a+b(1-e^{-ct})$ where p is DM and CP degradability (%) at time t,a is soluble fraction , b is degradable fraction , e is logaritm number, c is fractional rate and t is time of incubation.The data were analyzed using the General Linear Model (GLM) procedure of SAS Institute Inc (1987) which uses least square means for each parameter.Feeds were the only source of variation considered.

**Results** The DM content of alfalfa and red clover hays used in this experiment ranged from 900 to 920 $gkg^{-1}$ and 890 to 930 $gkg^{-1}$ and the CP content from 200 to 220 $gkg^{-1}$ and 150 to 170 $gkg^{-1}$ respectively.Alfalfa showed less variation than the other forage in the soluble DM fraction (Table 1) . Alfalfa hays soluble DM fraction had a standard deviation of 25 whereas red clover hays had a standard devation of 30.Degradable fraction B DM values behaved in a similar fashion to the soluble fraction A values with the alfalfa hays less variation than the red clover hays (Table1) . The soluble CP fraction A values for the alfalfa hays was much more than the red clover hays. Degradable fraction B CP values for the alfalfa hays were much less than for the red clover hays. The effective degradability of DM in the two types of forages had significantly differences ($p<.05$) between samples. The soluble and the degradable DM values of alfalfa hays observed in this experiment (Table 1) were lower than that reported by Mir et al (1991) . The soluble and the degradable CP red clover hays observed in this study (Table 1) were lower and more than from that reported NRC (2001) respectively. These differences may be a result of differences in growing condition and on individual farms.

**Table 1** Dry matter and crude protein degredation charateristics

| | DM | | | | CP | | | |
|---|---|---|---|---|---|---|---|---|
| Alfalfa hays(n=10) | Minimum | maximum | SD [1] | Significant | Minimum | maximum | SD | Significantt |
| Fraction A (g/kg) | 300 | 350 | 25 | - | 380 | 450 | 25 | - |
| Fraction B (g/kg) | 450 | 520 | 28 | - | 420 | 500 | 20 | - |
| EPD[2] (g/kg) | 300 | 400 | 18 | * | 400 | 520 | 27 | - |
| C ($gkg^{-1}h^{-1}$) | 26 | 29 | 3 | - | 50 | 61 | 5 | - |
| Red Clover hays(n=10) | | | | | | | | |
| Fraction A (g/kg) | 200 | 420 | 30 | - | 300 | 350 | 20 | - |
| Fraction B (g/kg) | 390 | 450 | 50 | - | 610 | 660 | 31 | - |
| EPD (g/kg) | 400 | 480 | 20 | * | 420 | 480 | 26.7 | - |
| C ( $gkg^{-1}h^{-1}$ ) | 30 | 36 | 1.5 | - | 12 | 20 | 6 | - |

1- Standard deviation 2-Effective degradability

**Conclusion** The overall results showed evidence of varietal as well as species differences in quality of forages.It is evident that, in the case of forages, the present use of average values for forages by the Feed Manufacturing Industry can lead to inaccurate feed formulation since they may not reflect the particular forage being used in the ration.

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# Apparent digestibility, rumen fermentation characteristics and microbial N entering duodenum in Iranian Baloochi Lambs fed diets formulated to contain similar amounts of ERDP with different protein sources

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**Introduction** Published results in the literature indicate that the inclusion of increased level of degradable protein in the ruminant diet resulted in a stimulation of ruminal pH, ruminal N-$NH_3$ and microbial N entering duodenum (Mesgeran & Parker, 1998). The objective of this experiment was to investigate the effect of altering the dietary sources of protein with similar amounts of ERDP on the apparent digestibility, ruminal fermentation characteristics and microbial protein entering duodenum in Iranian Baloochi lambs.

**Material and Methods** Four Iranian Baloochi lambs weighing 33 ± 1.3 kg, each with a permanent rumen fistula, were fed twice daily with diets differing in protein sources in a 4x4 Latin Square design. The diets consisted of a basal diet of chopped lucerne, barley and sugar beet pulp (190, 230 and 170 g DM $d^{-1}$, respectively) which was supplemented with lucerne (L), cottonseed meal (C), soybean meal (S) or molasses + urea (M+U) (210, 118, 84 and 80+9 g DM $d^{-1}$, respectively). The diets provided similar ERDP (87 g $kg^{-1}$ DM). Samples of rumen fluid were collected at 0.0, 1, 2, 4 and 6 hours after the morning feed. The pH of the rumen fluid was measured directly. The N-$NH_3$ concentrations were determined in deproteinised (2 ml rumen fluid + 2 ml 0.2 M HCl) samples using a colourimetry procedure. Urinary purine derivatives, as indicator of microbial N entering duodenum, were determined in the urine samples using the HPLC method of Mesgaran & Parker (1998). In order to determine the apparent whole tract digestibility, total fecal was weighed and sampled daily for 8 days in each period. The standard methods were used for all laboratory analysis.

**Results** The apparent digestibility results are presented in Table 1. The data indicate that the whole tract digestibility of DM, OM and CP were significantly effected by the protein sources ($P < 0.05$). Microbial N concentrations entering duodenum for L, S, C and M + U were 9.1, 9.8, 9.7 and 8.1 (SE: 1.5) g $d^{-1}$, respectively, while there were no treatment effect. The data related to ruminal pH and N–$NH_3$ concentrations are shown in Table 2.

**Table** 1 Apparent digestibility (g $kg^{-1}$)

| | Treatments * | | | | SEM | *P-Value* |
|---|---|---|---|---|---|---|
| | L | S | C | M+U | | |
| Dry matter | 737[a] | 800[b] | 768[c] | 809[b] | 5.6 | 0.01 |
| Organic matter | 758[a] | 820[b] | 790[c] | 829[b] | 5.4 | 0.01 |
| Crude protein | 622 | 693 | 647 | 700 | 16.0 | 0.06 |
| NDF | 750[a] | 801[b] | 759[a] | 803[b] | 11.4 | 0.04 |

***** Means on the same row with different superscripts significantly differ

**Table 2** Rumen fermentation characteristics

| Time (h) | | Treatments | | | | SEM | *P-Value* |
|---|---|---|---|---|---|---|---|
| | | L | S | C | M+U | | |
| 0.0 | pH | 6.80 | 6.69 | 6.17 | 6.48 | 0.17 | 0.13 |
| | N-$NH_3$ | 17.48 | 18.04 | 14.91 | 12.06 | 3.06 | 0.65 |
| 1.0 | pH | 6.19 | 6.13 | 5.85 | 6.33 | 0.09 | 0.10 |
| | N-$NH_3$ | 20.71[a] | 21.38[a] | 17.58[a] | 40.39[b] | 2.57 | 0.01 |
| 2.0 | pH | 6.09 | 5.85 | 5.65 | 6.07 | 0.09 | 0.90 |
| | N-$NH_3$ | 18.13[a] | 19.98[a] | 17.90[a] | 33.06[b] | 2.70 | 0.04 |
| 4.0 | pH | 6.21 | 5.89 | 5.72 | 5.92 | 0.12 | 0.13 |
| | N-$NH_3$ | 13.72[a] | 15.93[a] | 15.70[a] | 22.36[b] | 2.57 | 0.03 |
| 6.0 | pH | 6.46 | 6.12 | 5.89 | 6.20 | 0.13 | 0.11 |
| | N-$NH_3$ | 12.48 | 14.93 | 12.14 | 11.98 | 2.29 | 0.79 |

***** Means on the same row with different superscripts significantly differ

**Conclusion** The rumen pH was unaffected by diets. However, diets containing S and C resulted in a marked decreased in rumen pH in all sampling times. It is interesting to note that in the present study the ruminal N-$NH_3$ of lambs fed diets containing M+U was markedly higher compared to the other protein sources in the samples taken at 1, 2 and 4 hours after the feeding. Thus, it is agreed that the inclusion of a protein source with more quickly degradable fraction in the diets, even with similar ERDP, may contribute to the such changes after feeding.

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# Ruminal peptide and soluble protein N concentrations in Iranian Baloochi lambs fed diets containing lucerne hay or silage

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**Introduction** It is generally agreed that peptides accumulate in the rumen fluid after feeding. It has been also suggested that the production of peptides in the rumen was not altered by the protein supplements when diets provided similar effective rumen degradable protein (Mesgaran & Moosavi, 1999). The objective of the present experiment was to investigate the effect of diets containing lucerne hay or silage treated by urea and formaldehyde on the ruminal peptide and soluble protein N concentrations in Iranian Baloochi lambs.

**Materials and Methods** Four Iranian Balochi lambs weighing 33.7 ± 0.34 Kg, each with a permanent rumen fistula, were assigned to a balanced $4^2$ Latin Squares and fed as TMR twice daily. The diets consisted of a basal diet of ground barely and sugar beet pulp (180 and 120 g DM $d^{-1}$, respectively) which was supplemented with 300 g DM $d^{-1}$ lucerne hay (LH), lucerne silage (LS1), lucerne silage treated with 0.5 g urea and 0.4 g formaldehyde (LS2) or lucerne silage treated with 1 g urea and 0.4 g formaldehyde, per 100g DM (LS3). The diets provided similar ERDP (98.4 g $Kg^{-1}$ DM). Samples of rumen contents were taken, by suction, at 0.0, 0.5, 1, 2, 3 and 5 hours after the morning feed. Ruminal fluid was prepared for peptide analysis using sulphate-tungstate method described by Chen et al. (1987). The percoloric and tugstate acid-precipitate nitrogens were assayed by a standard macro-Kjeldahl procedure. The data were analysed using a repeated measures design; with sheep as a block, the effect of treatments repeated on time was tested against sheep x treatment and the effect of time was tested against the residual.

**Results** The peptide and soluble protein N concentrations at each sampling time are shown in the Table. The results showed no significant differences betwean the experimental groups. Howevers, the time effect was significant ($p < 0.01$). The ruminal soluble protein N concentrations in the lambs fed diet containing LH were generaly higher than the other groups in each sampling time. Peptide N concentrations increased after the feeding and declined at 5 hours after that, whereas the soluble protein N concentrations were notably decreased after feeding.

**Table1** Peptide and soluble protein N concentrations (mg $liter^{-1}$) in the rumen fluid of Iranian Balochi lambs fed the diets containing lucerne hay or silage

| Nitrogen fraction | Time(h) | Treatments | | | | Treatment effect | | Time effect | |
|---|---|---|---|---|---|---|---|---|---|
| | | LH | LS1 | LS2 | LS3 | SEM | Statistical significant | SEM | Statistical significant |
| Peptid N | 0.0 | 28.5 | 54.65 | 12.05 | 35.05 | 16.97 | NS | 13.84 | ** |
| | 0.5 | 67.75 | 79.13 | 118.38 | 112.95 | | | | |
| | 1 | 105.15 | 106.70 | 97.28 | 147.35 | | | | |
| | 2 | 116.40 | 126.33 | 111.60 | 121.98 | | | | |
| | 3 | 152.68 | 134.73 | 112.33 | 127.97 | | | | |
| | 5 | 112.78 | 53.15 | 105.03 | 106.07 | | | | |
| Soluble protein N | 0.0 | 275.75 | 289.58 | 219.70 | 255.00 | 33.26 | NS | 29.36 | ** |
| | 0.5 | 134.13 | 97.20 | 99.53 | 81.38 | | | | |
| | 1 | 133.00 | 71.80 | 91.45 | 100.85 | | | | |
| | 2 | 118.90 | 52.43 | 48.05 | 39.35 | | | | |
| | 3 | 141.50 | 22.18 | 47.45 | 48.45 | | | | |
| | 5 | 207.98 | 70.33 | 91.08 | 61.03 | | | | |

* * $p < 0.01$

**Conclusion** The data related to the present study showed that the ensiling of lucerne did not significantly influence the nitrogen metabolism in the rumen. Soluble protein N concentrations in the rumen fluid of lambs fed diet containing LH compared to other groups confirmed that lucerne hay protein was more susceptible to rumen degradation. Peptide N concentrations increased from 0.5 hour after the feeding and declined at 5 hours after that. However, the concentrations of peptide N at 5 hours after the feeding were still considerably higher than those of before feeding. So, from the physiological aspects, the determination of the composition of such peptides may be important in ruminant nutrition.

**Acknowledgement** The authors wish to acknowledge the funding for this work from the University of Mashhad, Iran.

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# Degradability characteristics and Intestinal protein apparent digestibility of Iranian soybean and cottonseed meals as assessed by the mobile nylon bag technique

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**Introduction** Ruminal degradability of feed protein is an important factor when assessing feed protein value according to the modern ruminant feed evaluation systems. In addition, the worth of any food protein to ruminant animals depends on the how the protein is digested in the small intestine. The work described in this summary assessed the in situ degradability and intestinal apparent digestibility of dry matter and protein, using ruminal and intestinal mobile nylon bags technique, of Iranian soybean (S) and cottonseed (C) meals.

**Materials and methods** The experimental feeds were heat extracted S and C. They originated from the Iranian varieties. Four Holstein steers (400 kg) Fitted with rumen fistula and T-shaped cannulae were used in the present study. For the rumen degradation studies, the experimental samples were milled (2 mm mesh) and weighed (5 g D M) into bags (12 x 19 cm) made of polyester cloth with 50 µm pore size (8 bags per each sample). The bags were incubated in the rumen for 0.0 , 2, 4, 8, 16, 24 and 48 hours. The ruminal, post ruminal and total tract disappearance of dry matter and protein of samples were determined using the mobile nylon bag procedure (De Boer et al., 1987). The bags (3 x 6 cm) were made of Dacron cloth with a pore size of 50 µm About 1.2 g dry matter of each protein sources (grounded through 2 mm screen) was placed in each bag (16 bags per each protein source), then inserted into plastic mesh cylinders (26 x 8 c m, 0.57 mm pore size ) and incubated in the rumen for 12 h. After removal from the rumen the bags were washed using cold water and those used to determine intestinal apparent digestibility (8 bags per each protein source) were incubated in pepsin-HCl solution (Subuh et al., 1996). The bags were then inserted into the small intestine via the canulae at the rate of one bag every 30 min and removed from the voided faeces, rinsed in cold ruining water. Finally, the bags were dried in a forced air oven (58° C ,for 24) and then weighted to determine the dry matter disappearance. The kjeldhal technique used for N analysis. The equation of $P = a + b ( 1 - e^{-ct})$ was used for the in situ degradability. The calculations described by Subuh et al. (1996) used for ruminal, intestinal and total tract disappearance of dry matter and Protein. Data were analysed using the GLM procedure of SAS.

**Results** In situ protein rumen degradation characteristics (a, b, c) for S and C were 0.49, 0.39 ,0.14 and 0.33, 0.54, 0.09, respectively. The data related to disappearance of DM and protein from mobile bags within rumen, intestine and total tract are shown in the Table.

**Table** Disappearance (g $Kg^{-1}$) of Iranian soybean and cottonseed meals from mobile nylon bags during incubation in the rumen and passage through the intestine

| | Soybean meal | Cottonseed meal | SE | statistical significant |
|---|---|---|---|---|
| **Intact feed -disappearance in the rumen** | | | | |
| DM | 736 | 455 | 19.1 | * |
| Protein | 602 | 436 | 115.8 | * |
| **Rumen undegraded -disappearance in the intestine** | | | | |
| DM | 762 | 231 | 31.2 | * |
| Protein | 237 | 792 | 24.3 | * |
| **Intact feed - disappearance in total tract** | | | | |
| DM | 938 | 575 | 22.9 | * |
| Protein | 975 | 880 | 21.1 | * |

** $P < 0.05$*

**Conclusion** the rumen degradable characteristics of soybean meal were considerably differed from those of cottonseed meal. The ruminal and post-ruminal DM and protein disappearances of soybean meal were also significantly higher compared to those of cottonseed meal. These difference agreed with the finding of the other workers and may be due to the chemical condition of these protein sources.

**Acknowledgement** The author wishes to acknowledge from the university of Mashhad, Iran**.**

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# The influence of chemical treatment on the degradation characteristics of weathered maize stover components

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**Introduction** Zambian small-holders are dependent on natural grazing to supply the nutrient requirements of their cattle. During the dry season grazing is severely limited, production declines steeply, reproductive cycles become dormant and new-born calves are subject to a high mortality rate. A potential supplemental feed, maize stover, is poorly exploited resulting in extensive field losses. Collection and controlled feeding would greatly increase its efficiency of use, plus such a feed system offers the opportunity to chemically treat the maize stover pre-feeding. This study was conducted to identify the degree to which the nutritive value of this material could be improved using such techniques.

**Materials and methods** Due to problems obtaining Zambian maize stover, weathered material was obtained from a standing maize crop (Reading, UK; August 2001) that had remained uncut due to poor field conditions the previous harvest (October 2000). Hand separated samples of stem (S), leaf (L) and husk (H) were left untreated (C), or treated with either 30 or 60 g NaOH (N) or urea (U) per kg field dry material or 60 g of a 1:1 mix of NaOH and urea (UN). The treatments were applied in solution at the rate of 1:1 (w/v) and the samples stored at either 39°C (U treatments) or 20°C for 14 days. Prior to analysis using the Reading Pressure Technique (Mauricio *et al*., 1999) the samples were dried (65°C, 3h) to permit milling (2 mm screen). Three 1.0g replicates of each substrate were prepared, together with three negative controls, for each of the seven withdrawal periods. The rumen fluid inoculum was obtained pre-feeding from a dry cow offered a hay / grass silage ration. Gas production was assessed from head-space pressure readings obtained 14 times during the 96 h incubation period. Fermentation residues were recovered after 6, 12, 19, 24, 36, 48 and 96 h to estimate organic matter degradation (iOMD). Substrates were examined for nitrogen, ash, NDF and ADF content. Estimates of metabolizable energy (ME) for the various fractions were obtained from DOMD values using a factor of 0.14. (MAFF, 1984). These were then summed, weighted according to their proportion in stover (0.65 : 0.25 : 0.10 for stem, leaf and husk, respectively) to provide stover ME values. SAS GLM procedures were used to generate LS means and significances of difference.

**Results** All NaOH treated samples showed considerable mould growth, however none was found in any of the urea treatments. NaOH treatment increased ash content of the substrates while urea elevated crude protein contents. Cumulative gas release at 96 h for SC and LC were similar and both considerably lower than that of HC (158, 156 and 255 ml $g^{-1}$ OM, respectively). All treatments increased the extent of fermentation, as estimated by gas production. Gas production varied with application level, with the highest NaOH treatment (60N) producing the greatest increase (Table 1). Significant substrate effects were identified, with leaf (L) showing the greatest response to treatment. Similarly all treatments significantly improved iOMD. Values obtained 48 h post-inoculation identified the S fraction to be poorly degraded, relative to either H or L. Greatest improvements were obtained from the application of 60 g $kg^{-1}$ NaOH, with the combination treatment showing the next largest effect. In addition all treatments improved fermentation efficiency (iOMD / gas). As the *in vitro* incubation medium used contained sufficient nitrogen to ensure that fermentation was not

**Table 1**: Influence of treatment on gas release, degradability (iOMD) and metabolizable energy (ME) content

| Treatment | Gas (ml $g^{-1}$ OM) | | | iOMD (g $kg^{-1}$) | | | Fermentation efficiency | | | ME stover |
|---|---|---|---|---|---|---|---|---|---|---|
| | Leaf | Stem | Husk | Leaf | Stem | Husk | Leaf | Stem | Husk | (MJ $kg^{-1}$ DM) |
| C | 128c | 115c | 198c | 518e | 389c | 655d | 4.0 | 3.4 | 3.3 | 4.2 |
| 30U | 143bc | 128b | 216ab | 641d | 501b | 738c | 4.5 | 3.9 | 3.4 | 6.1 |
| 60U | 155ab | 130b | 218ab | 666cd | 509b | 757c | 4.3 | 3.9 | 3.5 | 5.6 |
| 30N | 125c | 113c | 207bc | 683c | 567ab | 761bc | 5.5 | 5.0 | 3.7 | 6.8 |
| 60N | 171a | 142a | 226a | 836a | 616a | 912a | 4.9 | 4.3 | 4.0 | 8.6 |
| UN | 156ab | 120bc | 219ab | 732b | 554ab | 782b | 4.7 | 4.6 | 3.6 | 7.1 |
| s.e. | 9.3 | 5.5 | 9.3 | 15.4 | 42.2 | 13.1 | - | - | - | - |

Means in columns without similar letters are significantly different (P>0.05)

limited, it is considered that while the NaOH feeds have the potential to be fermented, if offered in a protein deficient situation, rumen degradabilities may be less than that estimated here. ME estimates suggest that untreated stover had a value of 4.2 MJ ME kg $DM^{-1}$, and that this was increased up to 8.6 MJ by treatment. As livestock, to varying degrees, select feed it is probable that the ME value of actual stover consumed will be higher, suggesting that the native Zambian cattle may be able to satisfy their maintenance energy demands from treated stover.

**Conclusions** Chemical treatment of maize stover, together with controlled feeding, offers the potential to partially alleviate the severe production losses and high calf mortality associated with the decline in grazing quality during the dry season in Zambia. The economic gains from the adoption of such techniques will be considerable.

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# Nutritive value of sunflower as whole crop silage in Uruguay

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**Introduction** Shortages of feed resources often impose major constraints on the development of animal production in the tropics and sub-tropics. Considerable quantities of crop residues and agro - industrial by products are generated every year in most developing countries. Forage crops must have particular features to warrant their use in animal production and feeding systems. These are related to seasonality of yield and product quality (Wilkins, 2000). Silage crops can provide forage of high nutritive value and high yields per unit of land are desirable to diversify crop rotations and to allow production changes, which may improve yields for livestock. Sunflower (*Helianthus annus*, L.) is mostly grown for seed oil production where the seeds represent only about one third of the total dry matter content of the crop ranging from 3 to 4 ton ha $^{-1}$. Reports on the chemical composition of sunflower silage suggested that total digestible nutrients of sunflower were two-thirds of that of corn silage. These characteristics of sunflower show its great potential so as to include it as a forage crop. Sunflower is grown in Uruguay for seed oil production (approximately 150000 ha) showing great potential to be include in animal production systems as an alternative silage crop. The objective of the present work was to evaluate the nutritive value of sunflower as whole crop silage.

**Materials and Methods** Three sunflower whole plants materials were harvested during summer 1999 and 2000, at three physiological stages: when capitullum elongates (*CE*), at 50 per cent flowering (*50F*) and at physiological maturity (*M*), in a 3 (material) x 3 (physiological maturity) x 2 (plant densities) split-plot design with three replicates. A representative sample of fresh chopped material was taken at each physiological stage. Laboratory silos were made using PVC tubes (20-cm diameter, 50-cm length). Samples were dry in a force oven (65 $^{0}$C during 48 hours) and milled through a 1 mm screen using a Wiley mill (Arthur H. Thomas, USA). Crude protein (CP), dry matter (DM), ash were determined according AOAC (1990), and acid detergent fibre (ADF) and neutral detergent fibre (NDF) according to Goering and Van Soest procedures (1967). Ether extract was determined by petroleum ether extraction (AOAC, 1990).

**Results and discussion** Mean values and standard deviation for the chemical composition at three physiological stages are given in Table 1. Laboratory silos show good quality characteristics for both CE and 50F. In maturity high EE and pH was verified. No significant differences between physiological stage in ADF and ash were observed. Differences between physiological stage in pH, EE, DM, NDF and CP were observed in sunflower whole crop silage ($P<0.05$).

**Table1. Mean and standard deviation (in brackets) for chemical composition in whole sunflower crop silage (g kg$^{-1}$ DM).**

| | CE | 50F | M | P |
|---|---|---|---|---|
| DM | 153.1 (27) | 183.5 (26) | 327.9 (54) | < 0.05 |
| CP | 146.5 (20.5) | 131.5 (15.5) | 104.1 (13.7) | < 0.05 |
| ADF | 383.2 (34.3) | 374.1 (39.8) | 409.3 (73) | NS |
| NDF | 611.8 (36) | 590.3 (51.9) | 484.1 (51.3) | < 0.05 |
| Ash | 145.3(21.4) | 125.9 (15.3) | 121.1 (19.9) | NS |
| pH | 4.4 (0.8) | 4.5 (0.5) | 6.2 (0.5) | < 0.05. |
| EE | 28 (4.6) | 38.1 (12.2) | 157.5 (43.3) | < 0.05 |

**Conclusion** The results of the present study demonstrate the potential of sunflower to be used as whole crop silage in animal production systems at Uruguay. The best physiological stage for harvesting sunflower is about 50 per cent flowering, where both high DM yield and nutritive value were obtained.

**Acknowledgements**

The work was supported by The National Institute for Agricultural Research – Uruguay (INIA)

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# The degradation characteristics of three Sri Lankan rice straw cultivars, following treatment with urea, assessed using three *in vitro* techniques

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**Introduction** In 1999 85 % of the milk and milk products consumed in Sri Lanka were imported at a cost of over 7000 m rupees. While this appears to offer a major opportunity for the national herd to improve production, indiscriminate deforestation, reduction of farm size and increased use of agricultural land for crop production has tended to depress both cattle numbers and production. Poor nutritional status of the animals is the major limiting constraint, caused by the inadequate supply of quality feedstuffs and confounded by the lack of advice from the poorly supported agricultural extension service. In addition little detailed information exists concerning the nutritive value of the majority of Sri Lankan feeds. Three *in vitro* techniques – the Minson and McLeod (1972) version of Tilley and Terry (T&T), the modified ANKOM (ANK) batch culture technique (Mould and Nordheim, 1998) and the RPT methodology (Mauricio *et al*., 1999) were compared in an effort to identify a suitable system to investigate Sri Lankan feeds. The degradation characteristics of rice straw were investigated in this study as, while nearly three-quarters of Sri Lankan cattle and buffaloes are reared in arid zone where rice straw is the major crop residue, only a small proportion is offered as feed.

**Materials and methods** Rice varieties (BG 300, BG 353 and BG380) cultivated during the long wet season were field dried, chopped (5 cm) and either left untreated (U) or treated (T) with 60 g urea $kg^{-1}$ straw, applied in solution at the rate of 1:1 v/w. The material was hand-mixed and stored at 30 °C for 14 days in sealed containers. All six samples were then partly dried and rotor milled to pass a 2 mm screen. The feeds were analysed for N, ash, NDF and ADF content. The *in vitro* studies were conducted consecutively over a period of two weeks using rumen fluid obtained pre-feeding (07.00h) from a single dry cow offered a grass silage / hay based diet. The effect of variety and treatment was examined within each *in vitro* technique using a complete latin-square design. The T&T technique was run in duplicate both with (+) and without (-) the acid-pepsin stage. The ANK study compared two incubation bag pore sizes (40 and 60 µm) using 50 x 65 and 50 x 50 mm bags, respectively to provide a similar open area. Bags were removed after 12, 24, 48 and 96 h incubation to estimate dry matter degradation (iDMD). Gas release profiles (RPT) were developed from head-space gas pressure values at 2, 4, 6, 8, 10, 12, 15, 19, 24, 30, 36, 48, 72 and 96 h. Organic matter degradation (iOMD) and iDMD were estimated 6, 12, 19, 24, 36, 48, 72 and 96 h post-inoculation.

**Results** Urea treatment significantly increased straw N content from a mean of 7.3 to 15.0 g $kg^{-1}$ DM and both NDF and ADF levels (by 34 and 52 g $kg^{-1}$ DM, respectively). All techniques identified a significant difference between untreated varieties in terms of degradation and that urea treatment significantly (P>0.0001) improved degradation in all cases. Little difference in fermentation gas release was seen between varieties either treated or untreated until 12 h post-inoculation (Table 1). Thereafter treated samples fermented much more rapidly reaching peak rates of gas release nearly twice that of the untreated controls 24 h post-inoculation. Asymptote values were not reached by the end of the 96 h fermentation period. To enable a comparison between techniques iDMD 48 h values are given in Table 2. Treatment significantly improved iDMD (466 and 585 g $kg^{-1}$ DM for U and T, respectively). Slight, but significant differences in iDMD were identified between techniques, although all ranked feeds similarly. The slightly higher values for the ANK technique probably reflects the final washing phase and could be considered similar to the acid-pepsin effect (T&T – /+) in terms of removing microbial contamination. The GLM procedure of SAS was used for statistical evaluation of experimental data.

**Table 1** Rate of gas release (ml $h^{-1}$)

| Substrate | | Rate of gas release | | | |
|---|---|---|---|---|---|
| | | 12h | 24h | 36h | 48h |
| BG300 | U | 1.64d | 4.44e | 4.07c | 3.47a |
| | T | 2.12b | 7.61b | 4.77a | 3.20b |
| BG353 | U | 1.83cd | 3.62f | 3.14e | 2.63e |
| | T | 2.94a | 6.71c | 4.33b | 2.77d |
| BG380 | U | 2.01bc | 4.70d | 3.77d | 3.05c |
| | T | 2.85a | 7.96a | 4.62a | 2.88d |
| s.d. | | 0.598 | 0.454 | 0.316 | 0.198 |

**Table 2** Substrate DMD (g $kg^{-1}$) according to *in vitro* technique

| Technique | BG300 | | BG353 | | BG380 | |
|---|---|---|---|---|---|---|
| | U | T | U | T | U | T |
| ANK 40 | 519a | 661a | 489a | 638a | 477b | 643a |
| ANK 60 | 484b | 614b | 434c | 592b | 464bc | 621b |
| RPT | 469bc | 557c | 400d | 526d | 474d | 593c |
| T&T - | 458c | 570c | 416c | 541d | 441cd | 574d |
| T&T + | 526a | 610b | 456b | 564c | 522a | 600c |
| Mean | 488 | 594 | 434 | 561 | 477 | 600 |
| s.d. | 15.3 | 14.0 | 16.6 | 17.0 | 15.2 | 9.8 |

**Conclusion** While all the *in vitro* techniques used produced similar degradation values, the larger capacity and ability to estimate rate of fermentation simultaneously identifies the RPT system as the preferred methodology.

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# The nutritive value of sugar beet pulp treated with *Neurospora Sitophila*

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**Introduction** In Iran, the availability of the protein supplements resources for ruminant animals is low. Therefore, protein enrichment of fibrous substrates such as sugar beet pulp (SBP) could make this by-product to be used as a protein supplement for livestock. Several workers have studied the effect of different fungi on the concentration of crude protein in the SBP (Lena and Quaglia, 1992; Shojaosadati et al , 1999). However, very little information is available in the literature regarding the effect of *Neurospora Sitophila* (NS) fungi on the nutrients digestibility as well as the protein quality of SBP for ruminants. Therefore, this experiment was carried out to study the changes in the chemical composition, the digestibility of dry matter (DM) and organic matter (OM) and the protein degradability of SBP treated with NS.

**Materials and methods** *Neurospora Sitophila* (ATCC 36935) was maintained at 4$^{o}$ C on potato dextrose agar (PDA) slants. SBP was obtained from local factories, dried at 90$^{o}$ C over night and then hammer-milled and sieved to 60gauge mesh. Inoculum preparation and the medium for solid state fermentation were carried out according to Shojaosadati et al (1999). The nutritional value of the treated SBP (TSBP) and untreated SBP (USBP) was carried out as follow: The chemical analysis was measured according to AOAC (1990). The digestibilities of the DM (DMD) and OM (OMD) were estimated using Tilley and Terry (1963) method. Three fistulated cattles, which were fed at maintence level, were used to determine the degradability of DM (DM deg) and protein (CP deg) (AFRC, 1992). The data of TSBP (n=5) and USBP (n=5) were compared statistically using unpaired T test analysis.

**Results** Table 1 shows the results. In comparison to USBP, the DM content of TSBP has decreased significantly ($P<0.05$), whereas, OM has not affected. The concentration of crude protein, DMD, OMD, DM deg and CP deg were significantly ($P<0.01$) higher in the TSBP than USBP. Treating the SBP with NS has led to decrease the ADF and NDF content significantly ($P<0.01$) in comparison to untreated SBP.

**Table 1** The chemical analysis (%), DDM and DOM (%) and the degradation of DM (DM deg) and protein (CP deg).

| Items | USBP | TSBP | Significance |
|---|---|---|---|
| DM | 90.0 ±3.3 | 78.0 ±2.2 | * |
| OM | 94.4 ±1.0 | 93.7 ±0.5 | Ns |
| CP | 9.2 ±0.1 | 25.5 ±0.4 | ** |
| ADF | 30.0 ±0.2 | 25.4 ±0.3 | ** |
| NDF | 46.6 ±0.2 | 27.2 ±0.3 | ** |
| DMD | 65.8 ±0.8 | 82.4 ±1.0 | ** |
| OMD | 70.9 ±0.6 | 89.6 ±0.8 | ** |
| DM deg | 58.0 ±0.1. | 62.4 ±0.6 | ** |
| CP deg | 53.1 ±0.8 | 65.2 ±0.3 | ** |

**Conclusions** The high rate of growth of NS on SBP had led to inrease the CP concentration. Additionly, an improvement in the DMD, OMD, DM deg and CP deg were observed which may be due to the decrease of NDF in TSBP. Such product may be useful to be used as a cheap protein supplement for ruminant animals particularly for high producing dairy cows.

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# Effects of fungal treated wheat straw on Feed intake and growth of fattening lambs.

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**Introduction** White- rot fungi have been investigated for biological upgradation of cereal straws into livestock Feed by solid substrate fermentation (Tripathi et al. 1991). Studies showed that pleurotus sajor- cajo(PSC) grew well on wheat straw and improved nutritive value (Leng 1990), due to the presence of wide spectrum of extracellular hydrolytic and oxidative enzymes and high infiltration of the mycelium into the substrate (Kokhreidze et al,1993).The objective of this study was to examine the effect of treated wheat straw by PSC edible fungi on feed intake and daily gain of fattening male lambs.

**Material and methods** Thirty - six of Moghani male lambs with 36.03± 3.01 Kg initial live weight at the age of 250 ± 15 days were assigned in these studies. The duration of trial was 104 days. The experimental design was complete randomized block, with three rations, three replications and four animal units.
The lambs were fed with three isocaleric and isonitrogenous rations (A, B, C) that containing wheat straw (UWS) , mycelium PSC treated wheat straw (MWS) and PSC harvested spent wheat straw (SWS) that composition of diets has been showed in table 1 and were formulated based on NRC (1985) and Predicted ME contents 10.45 Mj ME/Kg DM and CP 130g/Kg DM. Daily live weight gain (LWG) per 21 days and feed intake per a week were measured. In vitro dry matter digestibility (IVDMD) UWS , M WS and SWS were determined.

**Table1.** Composition of experimental diets (g/Kg DM)

| Ingredients | A | B | C |
|---|---|---|---|
| UWS | 200 | --- | --- |
| MWS | -- | 260 | --- |
| SWS | --- | --- | 250 |
| Lucerne | 50 | 50 | 50 |
| Barely | 530 | 520 | 530 |
| Wheat Bran | 120 | 50 | 37 |
| Cottonseed meal | 90 | 110 | 123 |
| Vit & Min | 10 | 10 | 10 |

**Results** The IVDMD of MWS was improved, but there was no significant difference between UWS and SWS ($P< 0.05$) (Table 2). A significantly lower total DM intake ($P<0.05$) was observed in animals fed a diet containing UWS than those fed diets containing MWS and SWS. The lambs were fed rations containing MWS and SWS gained significantly more than lambs fed ration containing UWS ($P<0.05$), but there was no significant difference between the lambs fed diets containing MWS and SWS. Feed conversion ratio didn't significantly change with the straw treatment (Table 3).

**Table 2** IVDMD of straws

| Item | UWS | MWS | SWS | SE |
|---|---|---|---|---|
| IVDMD | $0.975^{a}$ | $0.587^{b}$ | $0.498^{a}$ | 0.006 |

**Table 3** Effect of different diets on total DM intake and live weight gain.

| Item | UWS diet | MWS diet | SWS diet | SE |
|---|---|---|---|---|
| Total DM intake (Kg/ days) | $1.36^{a}$ | $1.59^{b}$ | $1.69^{b}$ | 0.04 |
| Live weight gain (g/d) | $144.74^{a}$ | $169.55^{b}$ | $180.75^{b}$ | 4.46 |
| Feed Conversion(DMI/LWG) | $9.78^{a}$ | $9.56^{a}$ | $9.44^{a}$ | 0.28 |

**Conclusion** Culturing mycelium of PSC on wheat straw improves IVDMD. Utilization of MWS and SWS increase feed intake and LWG. Therefore , in practical terms it is possible to improve of cereal straws by the strategy of growing non-toxic fungi.

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# Nutritive value of *Agaricus bisporus* mushroom spent wheat straw as ruminant feed

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**Introduction** Since last decades, much interest has been evidenced for bioconversion of lignocellulosic materials such as production of edible mushroom. In Iran, the mushroom industry has been expanded during the last 20 years and currently more than 50000 tons of mushroom compost is produced annually by aerobic fermentation system. The compost remained after cropping of mushroom constitutes a potential pollutant and its disposal increases the production cost. This waste material is usually rich of microorganisms and extra cellular enzymes (Ball and Jacksa, 1995) and contains a high level of nitrogen, calcium, phosphorus and trace elements and more degradable than the original straw in the rumen (Zadrazil, 1997). However, there are limited information regarding the nutritive value and utilisation of the mushroom spent straw in animal nutrition. This experiment was conducted to study the nutritive value and acceptability of the *Agaricus bisporus* mushroom spent wheat straw, obtained from bag system mushroom growing in sheep nutrition.

**Materials and methods** A 4×4 change over design experiment consist of four treatments (diets) and four periods of time was conducted, using 8 mature male sheep (two animals per diet) with an average weight 38.5 ± 2.8 kg. The animals were kept in individual metabolic cages and allocated to four wheat straw based diets including: control 0.0 (I), 10 (II), 20 (III), and 30kg/100kg (IV) spent wheat straw (SPWS), respectively. The diets were formulated to provide maintenance requirements and offered *ad lib* as total mixed ration (TMR) at 08:0 and 16:30 h. Each period of feeding trial lasted for two weeks with the first week for adaptation and the second week for collection. Feed intake was recorded and the nutrient digestibilities were determined, using faecal collection method. Urine was collected, analysed and the nitrogen balance was estimated. Data were analysed for parametric statistics, including analyses of variance using GLM procedure of SAS.

**Results** Spent wheat straw contained considerably lower organic mater (OM), crude fibre (CF), nitrogen free extract (NFE) and neutral detergent fibre (NDF), but it contained higher crude protein (CP), acid detergent lignin (ADL), Ca and Phosphorous (P) in comparison to the original wheat straw (Table 1). The average intake of dry mater (DM) and OM of diet IV was significantly ($p<0.05$) lower than the other diets (Table 2). The intake of digestible dry mater (DDM) and digestible organic mater (DOM) of the diets III and IV were also lower ($p<0.05$). However, animals received diet IV showed the lowest amounts of nutrient intakes. The digestibility of DM, OM, CP, NDF and acid detergent fibre (ADF) of the diets were statistically ($p<0.05$) different (Table 2). Inclusion of SPWS up to 20kg/100kg of the diet did not affect the digestibility of DM, OM, CF, NDF and ADF, but diet containing 30kg/100kg SPWS showed a lower ($p<0.05$) digestibility of DM, OM, NDF and ADF. Digestibility of CP was decreased ($p<0.05$) in the diets III and IV, but the highest reduction of digestibility was observed in diet IV. The nitrogen balance was significantly ($p<0.05$) different among the treatments (Table 3). Amount of nitrogen retention was increased by including the SPWS at 10 or 20 kg/100kg in the diet, but it was decreased in diet IV that contained 30kg/100kg SPWS.

**Table 1.** Composition[#] of wheat straw and spent wheat straw

| | I | II | III | IV | s.e.m |
|---|---|---|---|---|---|
| Intake (g/ kg $BW^{.75}$) | | | | | |
| DM | 74 [a] | 74 [a] | 70 [a] | 57 [b] | 8.6 |
| OM | 63 [a] | 63 [a] | 58 [a] | 44 [b] | 6.7 |
| DDM | 36 [a] | 35 [ab] | 33 [b] | 23 [c] | 3.9 |
| DOM | 34 [a] | 33 [ab] | 31 [b] | 20 [c] | 4.2 |
| Digestibility (g/100g) | | | | | |
| DM | 49 [a] | 47 [a] | 47 [a] | 41 [b] | 3.5 |
| OM | 53 [a] | 51 [a] | 53 [a] | 46 [b] | 3.1 |
| CP | 50 [a] | 47 [a] | 38 [b] | 28 [c] | 5.8 |
| CF | 47 [a] | 49 [a] | 50 [a] | 46 [a] | 1.8 |
| NDF | 47 [a] | 48 [a] | 47 [a] | 43 [b] | 2.4 |
| ADF | 42 [a] | 42 [a] | 41 [a] | 35 [b] | 3.7 |

[#] Effects of diets on all variables were significant ($p<0.05$)

**Table 2.** Average Feed intake and digestibility[#]

| | OM | CF | CP | NFE | NDF | ADL | Ca | P |
|---|---|---|---|---|---|---|---|---|
| IWS | 91 | 43 | 3.1 | 43 | 78 | 9.5 | 0.8 | 0.02 |
| SPWS | 65 | 18 | 11 | 35 | 67 | 21 | 5.4 | 0.9 |

IWS = Initial wheat straw SPWS = Spent wheat straw
[#] = (g/100g DM)

**Table3.** Nitrogen balance (average per animal per day)[#]

| | N intake | N excretion (g/d) | | N retention | |
|---|---|---|---|---|---|
| Diet | (g/d) | Fecal | Urine | g/d | g/100g |
| I | 12.7 [a] | 6.4 [c] | 5.1 [a] | 1.2 [b] | 10.5 |
| II | 12.3 [a] | 7.4 [ab] | 3.6 [b] | 1.4 [a] | 11.2 |
| III | 12.2 [a] | 7.6 [a] | 3.4 [b] | 1.3 [ab] | 10.7 |
| IV | 9.6 [b] | 6.9 [b] | 3.3 [b] | -0.6 [c] | 00.0 |
| s.e.m | 1.6 | 0.6 | 0.8 | 0.3 | 3.8 |

[#] Effects of diets on all variables were significant ($p<0.05$)

**Conclusions** It can be concluded that *Agaricus bisporus* harvested spend wheat straw, obtained from bag cultivation system, contained considerable amount of nitrogen and may be used as a ruminant feed. However, its utilisation in the diets of ruminants is limited because of high mineral content, which may reduce its acceptability and nutrient balances. This experiment showed that inclusion of spent compost straw up to 20kg/100kg of the diet did not affect the nutrients intake, digestibility and nitrogen balance.

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# Rice water in feeding of Holstein dairy cattle

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**Introduction** Nearly 2.4 million tons of rice is consumed by Iranian(37kg/person/year). Generally, in rice cocking process the grain is boiled and drained and a large volume of rice water is produced. Rice water is content of 5%DM, 0.43% CP, 3.75% NFE and almost 2 Mcal/KgDM gross energy. This liquid as a product of drained processing can be utilized in animal feeding (Valizadeh et al. 2000).Two studies carried out to evaluated the differences levels of rice water and its effects on milk production , rumen and blood metabolites of dairy cows.

**Materials and methods** In experiment 1, ten Holstein dairy cows were selected in early lactation, divided in two groups with equal milk, body weight and age. The experiment was carried out in two 21d periods (14d for adaptation and 7d for sampling) and two treatments: 1) basal diet plus ad lib. tap water(control); 2) basal diet plus ad lib. rice water. In experiment 2, 12 Holstein dairy cows with equal milk, body weight and age were used in the study. The cows in treatments1, 2, 3 and 4 had 100,0.0(Control); 50,50; 25,75 and 0.0,100 % water and rice water, respectively. In two experiments a total mixed ration based on concentrate and forage (50:50 DM based) were offered throughout the study and had following composition: DM 889gr/kg. CP 161gr/kg, and NEl 1.65Mcal/kg DM.The main determined parameters were daily feed intake , chemical composition of feed and liquids , amount and composition of the produced fresh milk , pH , VFA and NH3-N of the rumen liquor, pH, BUN and some other characteristics of the blood samples which were taken on a regular basis.

**Results** In experiment 1, average of rice water intake was about 91 liter /day and was significantly lower than control. Rice water didn't significantly decreased total DMI (basal diet plus DM of rice water( 21.87 vs.22.42), both decreased intake from basal diet(21.87 vs. 18.32, almost 18.5%). However the amount of DM that was consumed from rice water compensated DMI from the basal diet (0.0 vs.4.1). Rice water contributed an average of 8 to 11.5% of the total DMI. Rice water consumption increased digestibility of DM and OM (73.49 vs. 77.92 and 75.57 vs. 77.92, respectively) and decreased acetate:propionate ratio in the rumen fluid and shift of VFA production to higher propionate concentration. There were no significant differences between the mean rumen pH of cows (6.71 vs. 6.55). Total concentration of VFA, NH3-N and blood parameters such an uric acid, pH, BUN, cholesterol and triglycerides concentrations were not affected by the treatments. Rice water consumption significantly decreased milk fat and increased lactose of milk (3.37 vs. 3.20 and 4.48 vs. 4.76, respectively).The cost of each Kg DMI in treatment 2 reduced by 16%. The cost reduction rates in case of each Kg milk yield and FCM were 16 and 14% for treatment 2.In experiment 2 rice water increased total DMI (basal diet plus DM of rice water), (21.9, 22.2, 22.4 and 22.5), but decreased intake from basal diet(21.9, 19.3, 18.5 and 17.2), also, liquid intake were 112, 116, 105 and106 liter/day in treatments 1, 2, 3 and 4, respectively. Rice water consumption significantly increased digestibility of DM(72.4, 74.2, 76.7 and 77.9) and OM(73.5, 71.3, 73.2 and72.9), but there were no significant differences in digestibility of CP (73.6, 74.9, 77.4 and 75.9) and CF(73.5, 71.3, 73.2 and72.9) between the treatments 1, 2, 3 and 4. Data on milk yield and it's composition are presented in table1.Data on rumen metabolites are presented in table2.Total concentration of VFA,NH3-N and blood parameters such an uric acid,pH,BUN,cholesterol and triglycerides concentration were not affected by the treatments.

Table 1. Milk yield (Kg) and its composition(%)

| | Treatments | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | SE |
| Milk yield | 27.7 | 26.5 | 28.2 | 27.4 | 0.95 |
| Fat | 3.36 | 3.34 | 3.26 | 3.12 | 0.11 |
| Protein | 3.48 | 3.41 | 3.49 | 3.52 | 0.05 |
| Lactose | 4.48 | 4.52 | 4.64 | 4.76 | 0.03 |

Table2.Rumen metabolites

| | Treatments | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | SE |
| pH | 6.8 | 65 | 6.62 | 6.54 | 0.14 |
| NH3-N(Mol/d) | 6.69 | 6.76 | 6.52 | 6.50 | 0.21 |
| Total VFA(Mol/d) | 62.93 | 61.2 | 62.7 | 63.1 | 4.01 |
| Acetate/Propionate | 264.2 | 242 | 221.1 | 210.9 | 18.1 |

**Conclusion** A significant reduction in DMI of basal diet following feeding rice water, probably due to nutrients contains of the consumed rice water. Rice water consumption increased digestibility of DM and OM, While can be related to high digestibility of rice water contents and its availability for rumen micro flora. Fat of milk was significantly reduced , manly because of the decrease in acetate:propionate ratio in rumen fluid and shift of VFA production to higher propionante concentration .Also increasing lactose in milk can be related to this concept .It can be concluded that utilization of rice water in feeding dairy cattle not only reduces their wasted and pollution problems but also help to provided least cost diet in Iranian dairy industries. The results of the present study demonstrates that the basal diet level of rice water in feeding Holstein dairy cattle 75% of liquid intake or rice water can be used that met 20% of total DMI. .

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# Voluntary feed intake in autumn calving continental x dairy suckler cows given a grass silage based diet *ad libitum*

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**Introduction** An understanding of the dry matter intake (DMI) capacity of suckler cows is crucial to the provision of adequate nutrition during lactation. However, quantitative data on the likely feed intake patterns of modern continental x dairy suckler cow genotypes is scarce. The objective of the current experiment was to determine voluntary DMI in Simmental x Holstein/Friesian (SIM) and Belgian Blue x Holstein/Friesian (BB) autumn calving suckler cows offered a grass silage based diet *ad libitum.*

**Materials and methods** 8 SIM and 8 BB multiparous autumn calving suckler cows were group housed in 2 sawdust bedded pens and fed individually through Calan-Broadbent gates. Four cows of each breed type were suckling calves sired by either Aberdeen Angus (AA – mean cow parity 4.6 years) or Charolais (CH – mean cow parity 4.6 years) sires. Each sire type / dam type interaction comprised 2 male and 2 female calves. The average day of lactation (DOL) at the start of the experiment was 57 (range 45-65) and cow liveweight (LW) and condition score (CS) were measured fortnightly throughout. The experiment was a continuous randomised block design consisting of two, 9 week periods and started on the 10th November 2000. During period 1 cows were offered 1.56 kg/head/day of a barley/soyabean meal based concentrate (DM: 834 g/kg; ME: 12.9 MJ/kg DM; CP: 169 g/kg DM) and given *ad libitum* access to 1st cut precision-chop grass silage (DM: 210 g/kg; ME: 10.4 MJ/kg DM; CP 135 g/kg DM). During period 2 the cows were offered the same grass silage *ad libitum* but no concentrate supplement was given. Daily DMI was determined for each cow on days 18-21, 39-42 and 60-63 (Period 1) and days 81-84, 102-105 and 122-126 (Period 2) of the experiment. Average LW, CS and DMI parameters for periods 1 and 2 were analysed by analysis of variance (ANOVA) and the quadratic relationships between DMI and DOL were also determined.

**Results** Period averages for LW, CS, concentrate DMI (CDMI), silage DMI (SDMI) and total DMI (TDMI) along with average DOL are given in Table 1. Data are presented for the sire type by cow type interaction (ie: AA/SIM, AA/BB, CH/SIM and CH/BB). Both SDMI and TDMI were significantly higher ($P<0.05$) for the CH/BB cows compared with the AA/SIM cows during period 1. However, no statistically significant differences were observed in any other parameter during either period 1 or period 2. The quadratic relationships between TDMI (g/kg $LW^{0.75}$) and DOL for all SIM (Figure 1) and all BB (Figure 2) cows are also shown below. For the period between 64 and 189 DOL, stage of lactation explained approximately 18% and 33% of the observed variation in TDMI for SIM and BB cows respectively.

**Table 1**. Average LW, CS and DMI parameters for continental x dairy suckler cows during periods 1 and 2.

| | Period 1 | | | | | | Period 2 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | AA/SIM | AA/BB | CH/SIM | CH/BB | sed | Sig | AA/SIM | AA/BB | CH/SIM | CH/BB | sed | Sig |
| LW (kg) | 601 | 607 | 639 | 640 | 21.1 | NS | 562 | 580 | 591 | 598 | 17.2 | NS |
| CS | 2.79 | 2.94 | 2.77 | 2.75 | 0.083 | NS | 2.60 | 2.88 | 2.66 | 2.66 | 0.124 | NS |
| DOL | 96 | 96 | 98 | 97 | 16.0 | NS | 158 | 158 | 161 | 159 | 4.4 | NS |
| CDMI (kg/d) | 1.3 | 1.3 | 1.3 | 1.3 | - | - | - | - | - | - | - | - |
| SDMI " | $10.5^{a}$ | $11.5^{ab}$ | $12.2^{ab}$ | $12.5^{b}$ | 0.84 | * | 9.4 | 10.7 | 10.8 | 11.4 | 1.19 | NS |
| TDMI " | $11.8^{a}$ | $12.8^{ab}$ | $13.5^{ab}$ | $13.9^{b}$ | 0.84 | * | 9.4 | 10.7 | 10.8 | 11.4 | 1.19 | NS |
| TDMI (g/kg LW) | 19.7 | 21.2 | 21.2 | 21.7 | 1.38 | NS | 16.8 | 18.6 | 18.3 | 18.9 | 2.04 | NS |
| TDMI(g/kg $LW^{0.75}$) | 97.5 | 105.0 | 106.5 | 108.9 | 6.61 | NS | 81.9 | 89.2 | 90.8 | 93.7 | 10.46 | NS |

For each period, values not sharing common superscripts differ significantly ($P<0.05$).

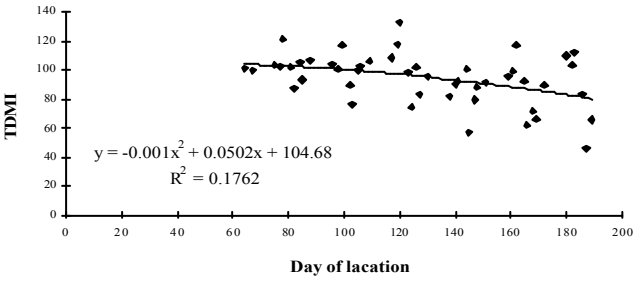


**Figure 1**. TDMI (g/kg $LW^{0.75}$) in all SIM cows

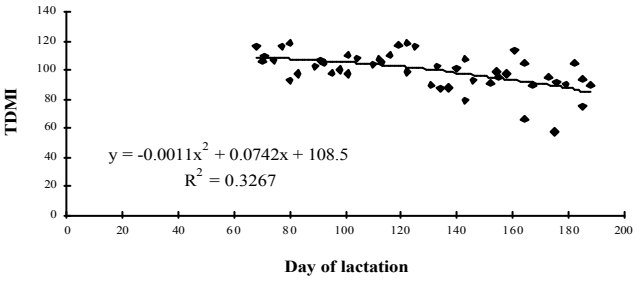


**Figure 2**. TDMI (g/kg $LW^{0.75}$) in all BB cows

**Conclusions** Continental x dairy cow breed (either SIM or BB) did not effect voluntary DMI in autumn calving suckler cows offered grass silage based diets *ad libitum.* There was some evidence to suggest that cows suckling calves sired by CH bulls consumed slightly more silage than cows suckling calves sired by AA bulls during the early winter feeding period. Voluntary DMI declined as lactation progressed through the winter in both cow types.

**Acknowledgements** This work was funded by DEFRA, MLC, Waitrose Ltd/Dovecote Park, the Aberdeen Angus Cattle Society and the British Belgian Blue Cattle Society.

# The effect of slaughter weight on growth and carcass traits of Holstein-Friesian bulls offered a cereal-based diet

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**Introduction** With the current situation of low profitability in the beef industry, producers must aim to produce beef efficiently and at minimum cost. In view of the ready availability of Holstein Friesian bull calves as a by-product of the dairy industry, the rearing and finishing of these animals offers a possible source of income to beef producers. The objective of the present study was to examine the influence of slaughter weight on feed efficiency and production characteristics of Holstein-Friesian bulls offered a cereal-based diet.

**Materials and methods** Fifty-four Holstein-Friesian bulls, mean initial age 171 (sd 8.2) days and live weight 230 (sd 23.4) kg, were used in a continuous, randomised block design experiment, with animals being slaughtered at one of four live weights: 400, 450, 500 or 550 kg. The animals were penned in groups of four and were offered concentrates *ad libitum* and a restricted quantity of barley straw (0.6 kg/head/d). Data on concentrate and total dry matter (DM) intake for each pen group were recorded daily throughout the trial. The concentrate portion of the diet contained fixed amounts (g/kg) of maize meal (100), sugar beet pulp (200) and a vitamin/mineral premix (25). The remainder of the concentrate consisted of barley and soyabean meal, with the proportion of the latter being reduced from 175 g/kg at the beginning of the trial to 120 g/kg at 350 kg live weight. The mean chemical composition of the overall diet offered throughout the study was: DM 867.5 g/kg, CP 174.4, MADF 110.0, NDF 229.4 and ash 75.1 g/kg DM. All bulls were weighed on two consecutive days initially and again prior to slaughter, and an extensive range of carcass measurements was recorded for all animals. Data on feed intakes and carcass parameters were analysed using the REML technique in Genstat 5 (release 4.1, Rothamsted, England). Live weight at the beginning of the experimental period and live weight gain from weaning to the initiation of the trial were used as covariates. Carcass weight at the beginning of the trial was estimated by assuming a dressing proportion of 0.47.

**Results** Treatment mean values for feed intake, feed conversion efficiency and carcass parameters are presented in Table 1. Daily feed intakes and total intake (concentrate plus straw) throughout the trial increased significantly ($P<0.001$) between the 400 and 550 kg slaughter weight groups. Bulls slaughtered at 550 kg remained on trial for 270 days compared with 134 days when slaughtered at 400 kg ($P<0.001$). Live weight gains throughout the trial were similar across treatment groups ($P>0.05$), but rate of carcass gain was significantly lower in animals slaughtered at 550 kg (0.70 kg/d) compared with those slaughtered at 400 kg live weight (0.78 kg/d) ($P<0.05$). Carcass weight increased by 83.9 kg, while feed conversion efficiencies decreased from 5.25 to 6.36 kg live weight/kg DM, and from 8.88 to 11.02 kg carcass/kg DM between the lowest and highest slaughter weight groups ($P<0.001$). Neither killing out proportion nor conformation grade was influenced by slaughter weight ($P>0.05$), but carcass fat grade was significantly lower in the 400 kg slaughter weight group than in any of the other weight groups ($P<0.001$). Marbling score increased significantly between 400 and 550 kg slaughter weight groups ($P<0.001$).

**Table 1** Data on feed intakes, animal performance and carcass parameters

| | Slaughter weight group (kg) | | | | sed | Sig. |
|---|---|---|---|---|---|---|
| | 400 | 450 | 500 | 550 | | |
| **Feed intake data** | | | | | | |
| Concentrate (kg DM/d) | 6.36 | 6.68 | 7.04 | 7.13 | 0.110 | *** |
| Straw (kg DM/d) | 0.45 | 0.49 | 0.51 | 0.55 | 0.009 | *** |
| Total (conc. + straw) on trial (kg DM) | 910 | 1286 | 1607 | 2067 | 51.2 | *** |
| **Animal performance and carcass data** | | | | | | |
| Days on trial | 134.2 | 179.5 | 213.1 | 269.7 | 53.98 | *** |
| Final live weight (kg) | 404.7 | 454.7 | 504.7 | 554.7 | 0.00 | *** |
| Live weight gain (kg/d) | 1.31 | 1.27 | 1.31 | 1.22 | 0.050 | NS |
| FCE live weight [1] | 5.25 | 5.70 | 5.83 | 6.36 | 0.219 | *** |
| Carcass weight (kg) | 211.9 | 239.4 | 267.3 | 295.8 | 2.79 | *** |
| Carcass gain (kg/d) | 0.78 | 0.74 | 0.76 | 0.70 | 0.033 | * |
| FCE carcass [1] | 8.88 | 9.74 | 10.10 | 11.02 | 0.453 | *** |
| Kill out (carcass weight/live weight) | 0.52 | 0.53 | 0.53 | 0.53 | 0.006 | NS |
| Conformation [2] | 3.57 | 3.10 | 3.92 | 3.56 | 0.499 | NS |
| Fat grade [3] | 3.44 | 4.77 | 4.64 | 4.67 | 0.320 | *** |
| Marble score [4] | 1.11 | 1.53 | 1.72 | 2.05 | 0.166 | *** |

[1] feed conversion efficiency (kg total DM/kg live weight or carcass gain); [2] 15 point scale: 1 = worst; 15 = best; [3] 10 point scale: 1 = leanest; 10 = fattest; [4] 8 point scale: 1 = low marbling; 8 = high marbling

**Conclusions** Efficiency of production (assessed as kg feed/unit gain) of Holstein-Friesian bulls declined significantly when slaughter weight was increased above 400 kg live weight. Furthermore, there was no benefit to carcass conformation by keeping bulls to higher live weights, although carcasses at very light weights were leaner. Cost of concentrate feedstuff and price/kg carcass received will be the key determinants of profitability.

# The effect of different levels of polyclonal antibody inclusion in a whey-based calf milk replacer on calf performances

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**Introduction:** The expectation in the EU is that the use of antibiotic growth promoters in animal production will be banned by 2005. The challenge is to find an appropriate alternative for use in calf milk replacer diets. Polyclonal antibody addition to the diet may offer a potential alternative to antibiotic feed additives. Surface active polyclonal egg antibodies derived from hyper-immunised chickens may offer such an alternative. Antibodies for common livestock pathogens can be raised in the hen and passed on to the egg and the eggs are used to produce hyper-immunised spray dried egg protein. However the optimum level of inclusion is not defined.

**Material and Methods:** Seventy two Friesian male calves were purchased from local farmers and auction markets at approximately 14 days of age (day 1) and randomly allocated on arrival to the following treatments.

1. polyclonal blend at standard level (std)
2. polyclonal blend at x4 standard level (std x 4)
3. polyclonal blend at x8 standard level (std x 8)
4. Polyclonal blend at x 32 standard level (std x 32)

The polyclonal antibodies were supplied by Trouw Nutrition, USA, the antibodies are effective against *Clostridium perfringens, E.coli* 987P*, E.coli* F4*, E.coli* K88*, E.coli* K99*, E.coli* J5*, S.typhimurium, S.cholarasuis, S.enteritidis, S.dublin, S.heidelberg,* Rotavirus and Coronavirus (TGE). The standard dose in the study was 125 ppm immunoglobulin inclusion. Calves were individually penned on straw throughout the 63 day experimental period. Each calf received a total of 25.5 kg of milk replacer over the 42 day replacer feeding period twice daily for first 28 day and once daily thereafter. The polyclonal blend was incorporated into the milk replacer which was reconstituted at the rate of 125 g powder made up to 1 litre by the addition of water and was offered warm (38$^{o}$C) by bucket over a 42 day period. A concentrate diet consisting (g/kg) of rolled barley (775), toasted extracted soyabean meal 200), mineral/vitamin (25) was available *ad libitum.* Clean fresh water was available at all times.

**Results:** There was a significant reduction in concentrate intake and liveweight gain when 32 times the standard level of polyclonal antibodies were added to the milk replacer (Table 1). Calves on the std x 8 treatment had a concentrate intake of 80.8 kg and a daily liveweight gain of 0.78 kg in the period 1 to 63 days. The corresponding values for std x 32 treatment were 58.7 and 0.60 kg respectively.This reduction was still evident in the period 43 to 63 days when the calves were weaned off milk replacer (Table 1).

**Table 1.** Effect of different levels of polyclonal antibody inclusion in a whey-based milk replacer on calf performance

| | Treatment | | | | | |
|---|---|---|---|---|---|---|
| | Std | Std x 4 | Std x 8 | Std x 32 | s.e.d. | Sign |
| Number of calves | 18 | 18 | 18 | 18 | | |
| Enteric disease[1] | 2 | 1 | 2 | 2 | | |
| Respiratory disease[2] | 13 | 12 | 10 | 16 | | |
| Concentrate Intake (kg) | | | | | | |
| 1-42 | 21.4$^{a}$ | 30.8$^{b}$ | 28.3$^{b}$ | 19.0$^{a}$ | 1.7 | * |
| 43-63 | 43.3$^{ab}$ | 49.6$^{b}$ | 52.5$^{b}$ | 39.7$^{a}$ | 3.3 | * |
| 1-63 | 64.7$^{ab}$ | 80.4$^{b}$ | 80.8$^{b}$ | 58.7$^{a}$ | 6.5 | * |
| Liveweight gain kg/day | | | | | | |
| 1-42 | 0.63 | 0.68 | 0.68 | 0.54 | 0.04 | NS |
| 43-64 | 0.77$^{ab}$ | 0.78$^{ab}$ | 0.92$^{b}$ | 0.72$^{a}$ | 0.05 | ** |
| 1-63 | 0.68$^{ab}$ | 0.72$^{ab}$ | 0.78$^{b}$ | 0.60$^{a}$ | 0.04 | ** |

Means with different superscripts differ significantly.
[1]two or more treatment courses
[2]more than two treatment courses

**Conclusion:** It was concluded that the new polyclonal preparation at 4 and 8 times the standard level gave the best calf growth rate response. In contrast the polyclonal antibody preparation at 32 times the standard level resulted in the lowest level of concentrate intake and the lowest liveweight gain.

# Carcass traits of Friesian, Piemontese x Friesian and Romagnola x Friesian steers finished on two feeding levels for two periods

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**Introduction** Crossing of Friesian-Holstein dairy cows with beef breed bulls is widely practised. Recently the Italian Piemontese and Romagnola beef breeds have been imported into Ireland, but they have not been evaluated. The objective of this study was to compare the progeny of these breeds and Friesians for carcass traits. General productivity traits have been reported elsewhere (Keane, 2001).

**Materials and methods** A total of 120 spring-born steers comprised of 40 Friesian-Holsteins (FR), 40 Piemontese x FR (PM) and 40 Romagnola x FR (RO) were reared together to slaughter at around 2 years of age. At the start of finishing they were blocked on weight to a 3 breed types (FR, PM, RO) x 2 feeding levels (3 kg (L) or 6 kg (H) per day of supplementary concentrates with grass silage *ad libitum)* x 2 finishing periods (124 (S) or 207 (E) days) factorial experiment. After slaughter carcasses were graded for conformation and fatness. After chilling, the right side was divided into a pistola hind quarter and fore quarter. The pistola was cut into 4 joints – distal pelvic limb, proximal pelvic limb, loin and ribs. These were separated into subcutaneous fat, intermuscular fat, muscle and bone. The data were analysed as a factorial with terms for block, breed, feeding level, finishing period and interactions.

**Results** There were no significant interactions of biological or practical importance. RO had a greater carcass weight than FR and PM which were similar (Table 1). All breeds differed significantly for kill-out. Conformation score was similar for PM and RO and fat score was similar for FR and RO with lower values for FR (conformation) and PM (fat score). The distribution of side weight between quarters and joints was similar for PM and RO but FR had lower proportions of pistola, proximal pelvic limb and loin than the beef crosses. All the breeds differed in pistola composition. FR had more bone and fat and less muscle than the beef crosses, while RO had more bone and fat and less muscle than PM. The H feeding level increased carcass weight, kill-out and conformation score but had only minor effects on the proportions of quarters and joints in the side and on side composition. The E finishing period increased carcass weight and fat score. It reduced the proportions of pistola and all pistola joints except ribs in the side and increased the proportion of fat and reduced the proportion of muscle in the pistola.

**Table 1** Slaughter traits and distribution of weights between quarters and tissues.

| | Breed type (B) | | | Feed level (F) | | Finishing (P) | | | Significance | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | FR | PM | RO | L | H | S | E | s.e.d[1] | B | F | P |
| Carcass weight (kg) | 324[a] | 326[a] | 341[b] | 321 | 330 | 311 | 350 | 7.7 | ** | *** | *** |
| Kill-out (g/kg) | 519[a] | 547[b] | 538[c] | 530 | 539 | 533 | 536 | 5.3 | *** | ** | NS |
| Conformation[2] | 2.08[a] | 2.88[b] | 2.90[b] | 2.43 | 2.80 | 2.65 | 2.58 | 0.096 | *** | *** | NS |
| Fat score[3] | 3.97[a] | 3.40[b] | 3.83[c] | 3.74 | 3.73 | 3.42 | 4.05 | 0.082 | *** | NS | *** |
| Proportions of side weight (g/kg) | | | | | | | | | | | |
| Pistola | 447[a] | 462[b] | 466[b] | 460 | 457 | 466 | 450 | 2.5 | *** | NS | *** |
| Distal pelvic limb | 53[ab] | 52[a] | 54[b] | 54 | 53 | 55 | 51 | 0.5 | * | * | *** |
| Proximal pelvic limb | 258[a] | 267[b] | 272[c] | 266 | 265 | 271 | 260 | 1.6 | *** | NS | *** |
| Loin | 81[a] | 86[b] | 85[b] | 84 | 84 | 85 | 83 | 0.8 | *** | NS | *** |
| Ribs | 53 | 54 | 52 | 54 | 52 | 53 | 53 | 1.1 | NS | NS | NS |
| Proportions of pistola weight (g/kg) | | | | | | | | | | | |
| Total bone | 193[a] | 169[b] | 176[c] | 183 | 176 | 180 | 179 | 2.4 | *** | *** | NS |
| Total muscle | 642[a] | 716[b] | 687[c] | 680 | 682 | 689 | 673 | 4.1 | *** | NS | *** |
| Total fat | 165[a] | 115[b] | 137[c] | 137 | 142 | 131 | 148 | 4.0 | *** | NS | *** |
| Discrete muscles | 479[a] | 536[b] | 519[c] | 510 | 513 | 516 | 506 | 3.5 | *** | NS | *** |
| Subcutaneous fat | 75[a] | 52[b] | 66[c] | 64 | 65 | 59 | 69 | 2.2 | *** | NS | *** |
| Intermuscular fat | 90[a] | 64[b] | 72[c] | 73 | 77 | 72 | 79 | 2.5 | *** | * | *** |

[1]For n = 40 (breed type). [a,b,c]Different superscripts indicate significantly different. [2]EU Beef Carcass Classification Scheme (Scale 1 = poorest to 5 = best). [3]EU Beef Carcass Classification Scheme (Scale 1 = leanest to 5 fattest).

**Conclusions** While RO had heavier carcasses than PM, muscle production was similar for both. There was little difference between PM and RO in the distribution of side weight between the quarters and joints. Higher feeding and longer finishing increased fatness and longer finishing also reduced the proportion of pistola in the side and the proportion of muscle in the pistola.

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# Piglet liveweight and rate of gain as predictors of body tissue composition

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**Introduction** The assumption that liveweight increase, decrease or stasis reflects piglet response to nutritional or management strategies may be incorrect. Adjustments in tissue composition following weaning result from altered metabolic state. Such compositional changes therefore accurately describe post-weaning response but are unlikely to be detectable by measurements of piglet weight alone. Accordingly, this study evaluates the accuracy of sequential weight measurements as predictors of tissue composition determined by dissection and chemical analysis. In addition, comparisons are made between weight measurement and body density (BD) predictions of tissue characteristics.

**Materials and methods** One hundred and sixty crossbred piglets (JSR Healthbred) were weaned from mixed parity sows at a mean age of 24.0 ± 0.24 days (± SEM) and weaning weight of 7.2 ± 0.10 kg. At weaning piglets were weighed, identified with an ear tag and housed in conventional, fully-slatted, flat-deck pens (1.99$m^2$). Feed and water were provided *ad libitum.* On day 20, 16 pigs were selected at random, weighed (LWT), killed and re-weighed underwater for determination of BD. Piglet average daily gain (ADG) was calculated. For tissue analysis the body was dissected into 4 fractions: head, legs and tail (HLT), thoracic and abdominal organs (ORG), carcass (CAR) and blood (data not shown). In addition, whole body composition (WBC) was calculated. Each fraction was frozen, minced, mixed and sub-sampled. Samples were analysed for dry matter (DM), ash and protein (N x 6.25). Lipid content was estimated by subtraction of ash and protein from total DM. LWT, ADG and BD were evaluated separately and in combination as predictors of tissue composition using the regression analysis procedures of Minitab 12.2.

**Results** Table 1 summarises the results of tissue and regression analysis for each body fraction. Mean values for day 20 LWT, ADG and BD were 12.7 ± 0.67kg, 0.259 ± 0.027kg and 1.0211 ± 0.001 respectively. With the exception of CAR, differences in fraction DM was more accurately explained by LWT than by BD, an effect most pronounced for ORG. Similarly, ORG protein and lipid content were less accurately predicted from BD than from a combination of LWT and ADG. CAR composition bore little relationship to ADG although increased DM and reduced protein content were associated with superior LWT. BD was the superior indicator of CAR tissue characteristics. Prediction of HLT protein and lipid were marginally more accurate for ADG than for BD, the reciprocal was true for the same tissues in WBC. Prediction equation accuracy was enhanced by combination of LWT and BD data (Table 1 bold script).

**Table 1** *Tissue composition and best regression predictors (P<0.05) for piglets 20 days post-weaning*

| | Dry matter (g/kg fresh weight) | | | | Protein (g/kg DM) | | | | Lipid (g/kg DM) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *value* | *Predictors* | *($R^2$)* | | *value* | *Predictors* | *($R^2$)* | | *value* | *Predictors* | *($R^2$)* | |
| CAR | 295 ± 8 | LWT<br>BD | (0.38)<br>(0.48) | **(0.66)** | 450 ± 13 | LWT<br>BD | (0.27)<br>(0.45) | | 521 ± 12 | BD | (0.48) | |
| HLT | 313 ± 6 | LWT<br>BD | (0.51)<br>(0.33) | **(0.64)** | 430 ± 8 | ADG<br>BD | (0.34)<br>(0.30) | **(0.58)** | 512 ± 19 | ADG<br>BD | (0.35)<br>(0.34) | **(0.63)** |
| ORG | 189 ± 4 | LWT | (0.78) | | 640 ± 12 | LWT + ADG<br>BD | (0.60)<br>(0.36) | | 347 ± 12 | LWT + ADG<br>BD | (0.60)<br>(0.36) | |
| WBC | 235 ± 6 | LWT<br>BD | (0.49)<br>(0.34) | **(0.63)** | 593 ± 8 | LWT<br>BD | (0.44)<br>(0.45) | **(0.68)** | 379 ± 8 | LWT<br>BD | (0.40)<br>(0.46) | **(0.65)** |

**Conclusions** With the exception of ORG, sequential weight measurements failed to predict whole body or body fraction tissue composition with greater than 49% accuracy. As such, reliance on traditional measures of piglet performance (LWT and ADG) as indicators of tissue flux and thus true piglet response post-weaning may be inadequate. BD prediction of tissue composition was generally less accurate than for LWT and ADG. In this experiment BD was calculated from under-water weight of the whole pig and was therefore undoubtedly compromised to some degree by the contents of respiratory and digestive tracts. However, combination of sequential weight and BD measurement data greatly improved prediction accuracy. This finding clearly indicates the potential for a practical and non-invasive method of body density measurement to support traditional sequential weight assessment of piglet response management and nutritional strategies.

# Effect of removing pigs from a pen at slaughter weight on the growth performance of the remaining animals

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**Introduction** When emptying finishing barns, it is common practice on U.S. operations to dispatch pigs over a two- to three-week period with the heaviest animals being selected first. However, there has been little research carried out under commercial conditions on the effect of removing pigs from a pen at slaughter weight on the performance of the remaining animals. This study was carried out to investigate the effect of removing the heaviest 30% of animals from a pen on the subsequent growth performance of the remaining pigs.

**Materials and methods** Two studies were carried out in two different wean-to-finish facilities. In Study 1, crossbred pigs (n = 648) were used to evaluate two removal strategies (Intact - no pigs removed, 24 -27 pigs/pen) vs (Reduced - ~30% removed leaving 18 pigs/pen]) on pig performance from wk 24 (time of removal) to day 12 post-removal. Floor and feeder space per pig were 0.64 $m^2$ and 3.4 cm and 0.96 $m^2$ and 5.1 cm for the Intact and Reduced treatments, respectively. In Study 2, crossbred pigs (n = 486) were used to evaluate two removal strategies (Intact - no pigs removed, 23-28 pigs/pen) vs (Reduced - ~30% removed leaving 18 pigs/pen) on pig performance from wk 24 (time of removal) to day 21 post-removal. Floor and feeder space per pig were approximately 0.60 $m^2$ and 2.3 cm and 0.90 $m^2$ and 3.4 cm for the Intact and Reduced treatments, respectively. Single-sex pens were used in Study 1 (n = 24) and mixed-sex pens (equal ratio of barrows to gilts) were used in Study 2 (n = 18). In both studies replicates were formed from pens of pigs which were paired on the basis of similar body weight, group size, and sex ratio. In both studies, pigs had free access to feed and water; feed intake data were only collected in Study 1.

**Results** Growth performance data for Studies 1 and 2 are given in Table 1. Two analysis were conducted. The first compared growth performances between the entire group of pigs after removal (Intact [24 - 27 pigs/pen]) vs (Reduced [18 pigs/pen]). The second analysis compared the lightest 18 pigs in both the Intact and Reduced treatment pens (Intact [18 lightest pigs/pen] vs (Reduced [18 pigs/pen]). In Study 1, for the entire pen of pigs feed intake was increased (8%; $P < 0.01$) and there was a numerical increase in growth rate (6%; $P = 0.227$) for pigs on the Reduced compared to the Intact treatment (Table 1). There was no treatment effect on gain:feed ratio. In study 2, growth rates were also higher (15%; $P < 0.05$) for the Reduced compared to the Intact treatments. Growth rates of the lightest 18 pigs in the Reduced and Intact treatment groups were 795 and 712 g/day (s.e.m. 22; $P < 0.05$) and 847 and 729 g/day (s.e.m. 33; $P < 0.05$) for studies 1 and 2 respectively. A treatment difference in the coefficient of variation for live weight at the time of removal was observed for Study 1 ($P < 0.01$) and Study 2 ($P < 0.05$), however, there were no differences for the coefficient of variation in weight over the remainder of the study (Table 1). No treatment differences in mortalities were observed in either study.

Table 1 Effect of pig removal on pig performance for the entire group for Studies 1 and 2

| | Studv 1 | | | Studv 2 | | |
|---|---|---|---|---|---|---|
| Item | Intact | Reduced | s.e.m. | Intact | Reduced | s.e.m. |
| Weight before removal, kg | 116.4 | 116.0 | 0.51 | 106.3 | 106.0 | 0.57 |
| Weight after removal, kg | 116.4 | 111.9 | 0.45*** | 106.3 | 102.0 | 0.41** |
| Weight at day 12 (Study 1) or 13 (Study 2), kg | 125.4 | 121.4 | 0.71* | 117.1 | 113.7 | 0.72* |
| Weight at day 21, kg | - | - | - | 121.5 | 119.9 | 0.98 |
| Daily gain,g (removal to end of study) | 749 | 795 | 23 | 719 | 847 | 36* |
| Daily feed intake, kg | 2.49 | 2.72 | 0.039** | - | - | - |
| Gain:feed | 0.30 | 0.29 | 0.011 | - | - | - |

*, **, *** = $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

**Conclusions** These results suggest that removing ~30% of the heaviest animals from a pen at slaughter weight will increase daily feed intake and improve average daily gain for the remaining pigs in the pen. Further research is needed to establish the effect of animal weight, timing, and the number of animals removed on subsequent performance.

# Effects of stocking rate and feeder-trough space on pig performance in wean-to-finish production systems

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**Introduction** Wean-to-finish production, which involves taking pigs from weaning to slaughter in the same building, is becoming widely adopted in the US swine industry. This production system is being advocated largely because of claims of improved animal performance and a decrease in labor needed for animal movement compared to conventional multiple-stage systems. One of the potential disadvantages of this system is the considerable underutilization of floor space during the early growth period if pigs are penned in the group sizes that are appropriate for finishing pigs. However, increasing the stocking rate initially at weaning, with some of these pigs subsequently being moved to another finishing facility, may increase output from the system. Therefore, the objective of this research was to investigate effects of initial stocking rate and of feeder-trough space in commercial wean-to-finish facilities on pig performance from weaning to slaughter.

**Materials and methods** Three studies were carried out in two different wean-to-finish barns. In Study 1, pigs (n = 1,560) were used to evaluate two initial stocking rate treatments (Single [52 pigs/pen] vs Double [104 pigs/pen]) on pig performance from weaning (5.9 ± 0.01 kg BW; 17 ± 2 d of age) to slaughter (114 ± 0.67 kg BW). Floor and feeder space per pig were 0.650 $m^2$ and 4 cm and 0.325 $m^2$ and 2 cm for the single- and double-stocked treatments, respectively. In Study 2, pigs (n = 1,458) were used to evaluate two initial stocking rate treatments (Single [27 pigs] vs Double [54 pigs]) on pig performance from weaning (4.8 ± 0.01 kg BW; 15 d of age) to slaughter (24 wk post-weaning). Floor and feeder space per pig were 0.640 $m^2$ and 3.4 cm and 0.320 $m^2$ and 1.7 cm for single- and double-stocked pens, respectively. In both studies, double-stocked pens were divided at the end of wk 10 into two equal-sized groups of similar mean BW and CV of BW, and one group was moved to a different pen in the same building. In Study 3, pigs (n = 1,728) were used to evaluate the effect of feeder-trough space (4 cm/pig [Increased] vs 2 cm/pig [Control]) on pig performance during the period of double stocking (to 8 wk post-weaning). Study 3 was conducted in the same barn as Study 1. In all studies, pigs had free access to feed and water; feed intake data were only collected for Studies 1 and 3. All data were submitted to an analysis of variance with the pen of pigs considered the experimental unit.

**Results** Mean growth performance data for Studies 1 and 2 are given in Table 1. For the first 10 wk post-weaning, Double compared to Single stocking resulted in lower ADG (7.7 and 7.9%, for Studies 1 and 2, respectively; $P < 0.001$), lighter pigs at end of 10 wk (6.8 and 7.3%, respectively; $P < 0.001$), and in Study 1, lower ADFI (7%; $P < 0.001$) but similar G/F ($P > 0.05$). From wk 11 to slaughter, pigs on Double and Single treatments had similar ($P > 0.05$) ADG in both studies, and in Study 1, ADFI was unaffected by stocking rate, but double-stocked pigs had improved G/F (4%, $P < 0.01$). Double-stocked pigs required an additional 2 days to reach a fixed slaughter BW ($P < 0.05$) in Study 1 and were lighter (4%; $P < 0.05$) at 24 wk post-weaning in Study 2. Carcass measures were similar ($P > 0.05$) for double- and single-stocked pigs. Double-stocked pigs that were moved at the end of 10 wk had similar ($P > 0.05$) growth performance to those that remained in their original pen. In study 3, doubling feeder-trough space did not affect ($P > 0.05$) pig growth performance from weaning to the end of wk 6. However, from wk 6 to 8, pigs on the Increased- compared to the Control-trough-space treatment had higher ADG (669 vs 633 g; $P < 0.05$) and were heavier (31.7 vs 30.9 kg; $P < 0.05$) at the end of wk 8, however, ADFI (1254 vs 1221 g) and G/F (0.53 vs 0.52) were not different ($P > 0.05$) for the two treatments from 6 to 8 wk.

**Table 1** Effect of stocking rate on pig growth performance for first 10 weeks post-weaning for Studies 1 and 2

| | Study 1 | | | Study 2 | | |
|---|---|---|---|---|---|---|
| Stocking rate | Double | Single | s.e.m. | Double | Single | s.e.m. |
| Weight at start of test, kg | 5.8 | 5.9 | 0.01 | 4.8 | 4.8 | 0.01 |
| Weight at end of wk 10, kg | 42.6 | 39.7 | 0.21[#] | 45.0 | 41.7 | 0.41[#] |
| Daily gain | 545 | 503 | 3.2[#] | 535 | 493 | 5.1[#] |
| Daily feed intake | 942 | 878 | 8.5[#] | - | - | - |
| Gain:feed | 0.57 | 0.58 | 0.003 | - | - | - |
| Removal rate, % | 2.1 | 2.1 | 0.69 | 2.3 | 2.0 | 0.61 |

[#] Effect of stocking rate was significant ($P < 0.001$)

**Conclusions** These results indicate that initial double stocking of wean-to-finish systems reduced growth rate to 10 wk post-weaning but in the subsequent period until slaughter had no effect on growth rate and resulted in greater feed efficiency. In addition, pigs that are double-stocked in wean-to-finish pens for longer than 6 wk post-weaning need additional feeder-trough space to maintain growth performance.

# The reproduction of *in sacco* degradation profiles using an *in vitro* batch culture technique

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**Introduction** The *in sacco* technique (e.g. Ørskov *et al*., 1980) remains the methodology with which the majority of rumen degradability estimates are made. However ethical, animal welfare and cost issues regarding the use of surgically modified animals plus the relatively slow rate at which samples can be examined (two samples per week using three sheep) have combined to compel researchers into developing alternative techniques. Precise, high capacity *in vitro* systems now exist. However the feed industry is unwilling to adopt these "new" values, partly due to having spent considerable time and effort creating databases of *in sacco* values which can be used directly in current feed evaluation systems. This paper describes modifications to an *in vitro* system that provides values similar to that obtained *in sacco*.

**Materials and methods** Both the studies presented here used the technique of Mould & Nordheim (1998), with the rumen fluid inoculum prepared using samples obtained pre-feeding (07.00h) from a dry cow offered a hay / grass silage / barley straw diet. In the first study 500 mg rotor-milled grass hay (1 and 2 mm screen) was incubated in either ANKOM F57 filter bags or bags prepared from monofilament polyester filter cloth (FC) with mesh sizes of 40, 50 and 60 µm. To ensure a similar open area, 50 x 65 and 50 x 50 mm bags were prepared (heat-sealed) using 60 and 40 / 50µm cloth, respectively. In the second study 50 µm bags were used, with 400 mg of each of four oat straws (OS1 – OS4), ground to 1 mm, incubated per bag. In both studies triplicate samples were used for each of the eight incubation periods (6, 12, 18, 24, 36, 48, 72 and 96 h). Dry matter degradabilities (DMD), respectively) were examined using SAS GLM procedures to generate LS means and significances of difference. Oat straw degradation profiles were compared to fitted *in sacco* values (Ørskov & MacDonald (1979) obtained in an earlier experiment.

**Results** A slight, but significant tendency for 1 mm hay to show a higher level of degradability compared to 2 mm was observed. This difference varied with pore size, but was found to be greatest with the ANK bags. The small difference in DMD between mesh size decreased over the incubation period, and at all incubation times DMD of the FC bags was significantly greater than that of the ANK bags (Table 1). In addition while the FC bags exhibited a typical sigmoidal degradation profile that of the ANK bags was irregular, tending to linear. The second study identified a highly significant relationship between the OS degradation profiles and the *in sacco* values (Figures 1 to 4). The marked discrepancy (0-18 h post inoculation) between the two sets of data is due to the inability of the model to accurately generate values at short time intervals, rather than to problems with the FC bags or methodology applied.

**Table 1:** Influence of bag type on DM degradability (g kg$^{-1}$)

| Bag | DMD hours post-inoculation | | | | | | |
|---|---|---|---|---|---|---|---|
| | 6 | 12 | 24 | 36 | 48 | 72 | 96 |
| 40 | 235a | 333a | 612a | 696a | 752a | 778a | 813a |
| 50 | 234a | 317a | 567b | 67ab | 734a | 773a | 814a |
| 60 | 238a | 293b | 542b | 644b | 717a | 767a | 809a |
| ANK | 215b | 231c | 319c | 406c | 492b | 513b | 693b |
| s.d. | 9.0 | 13.4 | 25.6 | 28.4 | 22.4 | 29.3 | 10.6 |

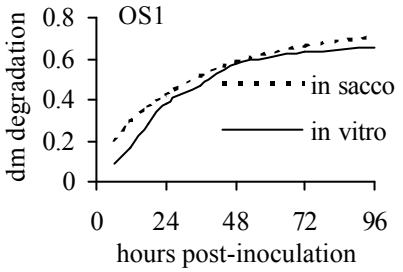


**Figure 1**: DM degradation (g g$^{-1}$)

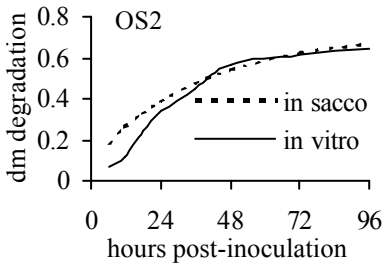


**Figure 2**: DM degradation (g g$^{-1}$)

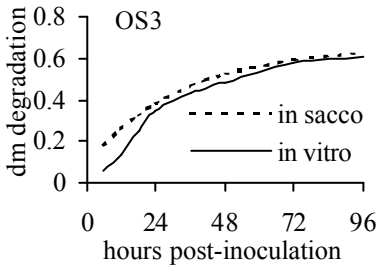


**Figure 3**: DM degradation (g g$^{-1}$)

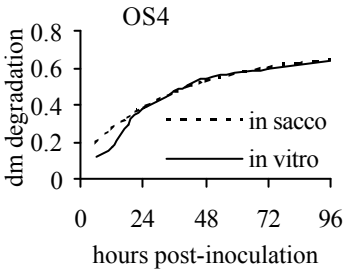


**Figure 4**: DM degradation (g g$^{-1}$)

**Conclusion** Not only does the modified technique provide an accurate estimate of *in sacco* degradability but has a much greater capacity. This offers to potential to utilise the technique in large-scale screening studies. As many feeds can be incubated simultaneously (in identical buffered rumen fluid), potential between / within animal errors are eliminated. A further advantage is that, being an *in vitro* system, the incubation environment can be exactly defined and many more experimental parameters investigated. Finally the ability to produce values similar to those obtained *in sacco* should help greatly in its acceptance and use by the feed industry.

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# A Rusitec based model of acute rumen acidosis: effects of nitrogen source on pH and feed degradation before and after glucose infusion

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**Introduction** Acute (clinical) and subclinical rumen acidosis is a problem in many production systems where readily fermentable concentrates are fed. In previous experiments (Haubi *et al*., 2001) a model for subclinical acidosis using the Rusitec was presented. The objective of the present study was to establish a model for clinical acidosis, where excessive carbohydrate fermentation drops rumen pH below 5.0. The effects of N source on pH and feed dry matter degradation (DMD) before and after an acute case of acidosis were determined by comparing level of diet N and source (non-protein nitrogen [NPN] versus soya protein).

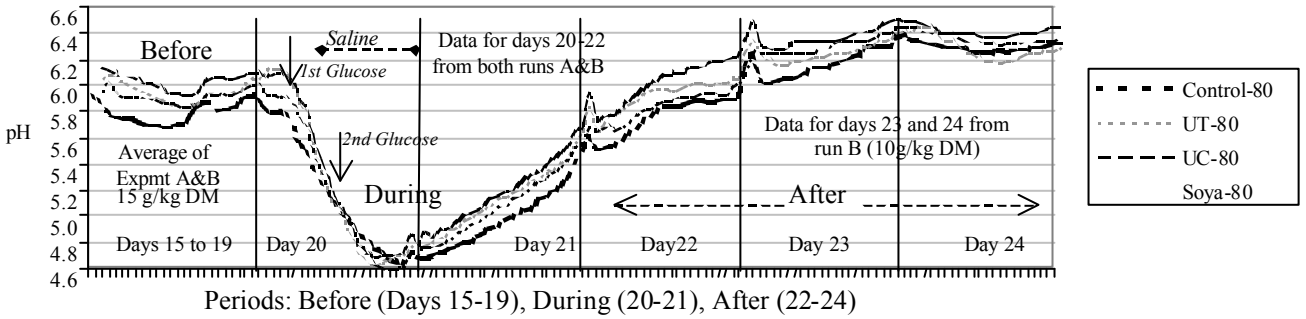
**Material and methods** Two 25-day Rusitec experiments (A and B) each with eight vessels were run using rumen fluid inoculum obtained from lactating cows on a high concentrate diet. Turnover of 0.75/day was established with 80% buffer. The 4 treatments examined (T1-T4) were 4 diets (15g DM/day) with different N sources. T1: Wheat (300g/kg DM) and maize silage (700g/kg DM) with a calculated crude protein (CP) of 100g/kg DM (Control). Diets T2 and T3 were modified with urea and sodium sulphate (12:1 ratio of N to S) to increase CP to 160g/kg DM. In T2 urea was given as a bolus (UB) and in T3 it was infused continuously with the buffer (UC). T4 (Soya) combined 300g wheat, 537g maize silage and 163g soya per kg DM (CP 160g/kg DM). Washed hay (300mg) was used as an assay for cell wall degradation. Acute acidosis was induced on day 20, by infusing 6g of glucose at 6 and 12 hours post-feeding. Saline replaced buffer infusion from 2.5 hours after the first glucose injection until the following feeding time. On day 1 of recovery feedbags were not replaced to mimic appetite depression following acidosis and buffer infusion was continued. In study A the diet was changed to alfalfa stems (15g DM) while in study B original diet was offered at a lower level (10g DM). Measurements and samples were taken before, during and after glucose (Figure 1). ANOVA procedures were used to test treatments effects within periods.

**Results** The model produced an acute acidosis (pH 4.82) followed by recovery. Daily gas production increased during acidosis due to extra glucose (Table 1). Negative gas production was observed after acute acidosis, which could be due to changes in fermentative pathways. Hay DMD dropped during acidosis and recovered within 3d, although recovery was slower for T1. Time below pH 6.0 before glucose ranked (T1>T4>T3>T2) possibly due to ammonia buffering.

**Table 1**. *Effects of nitrogen source on pH at feeding time, hay DMD, time below pH 6.0 and gas production.*

| Treatments | pH at feeding time | | | Hay DMD [g/kg] | | | Time pH < 6.0 [min] | | | Gas-production [ml] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Before | During | After | Before | During | After | Before | During | After | Before | During | After |
| Control | $6.00^{a}$ | $4.77^{a}$ | $6.52^{a}$ | $86^{a}$ | $28^{a}$ | $50^{a}$ | $978^{a}$ | $1280^{a}$ | $0.0^{a}$ | $300^{ab}$ | $1560^{a}$ | $24^{ab}$ |
| UB | $6.12^{b}$ | $4.83^{a}$ | $6.52^{a}$ | $187^{b}$ | $7^{b}$ | $194^{b}$ | $272^{b}$ | $1126^{b}$ | $48.7^{a}$ | $237^{a}$ | $1943^{a}$ | $17^{ab}$ |
| UC | $6.20^{b}$ | $4.88^{a}$ | $6.64^{b}$ | $107^{a}$ | $8^{b}$ | $159^{b}$ | $312^{b}$ | $1150^{bc}$ | $0.0^{a}$ | $264^{ab}$ | $2187^{a}$ | $-47^{a}$ |
| Soya | $6.21^{b}$ | $4.81^{a}$ | $6.59^{bc}$ | $141^{ab}$ | $16^{ab}$ | $116^{ab}$ | $549^{b}$ | $1230^{ac}$ | $0.0^{a}$ | $328^{b}$ | $1881^{a}$ | $45^{b}$ |
| s.e. | 0.02 | 0.06 | 0.03 | 22.5 | 5.1 | 25.2 | 100.7 | 22.2 | .029 | 25.0 | 176.1 | 25.8. |

LS Means in columns without similar letters are significantly different (P>0.05)




Periods: Before (Days 15-19), During (20-21), After (22-24)

**Figure 1**: *Daily pH variation before, during and after the induced acidosis*

**Conclusions** Although ruminal acidosis can depress fiber DMD, fibrolytic activity appears to recover if an adequate dose of buffering is given and a period of no or low level of feeding is allowed. Higher levels of NPN or true protein may be useful to reduce the time of recovery. This Rusitec model could be used to evaluate prophylactic and clinical treatment of acidosis with ionophores, probiotics and other commercial compounds.

**Acknowledgements**. C.U.Haubi would like to thank the National Council of Science and Technology of Mexico (CONACYT) for financial asisistance.

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# Fresh or frozen rumen contents as sources of inocula to estimate in vitro degradation of ruminant feeds

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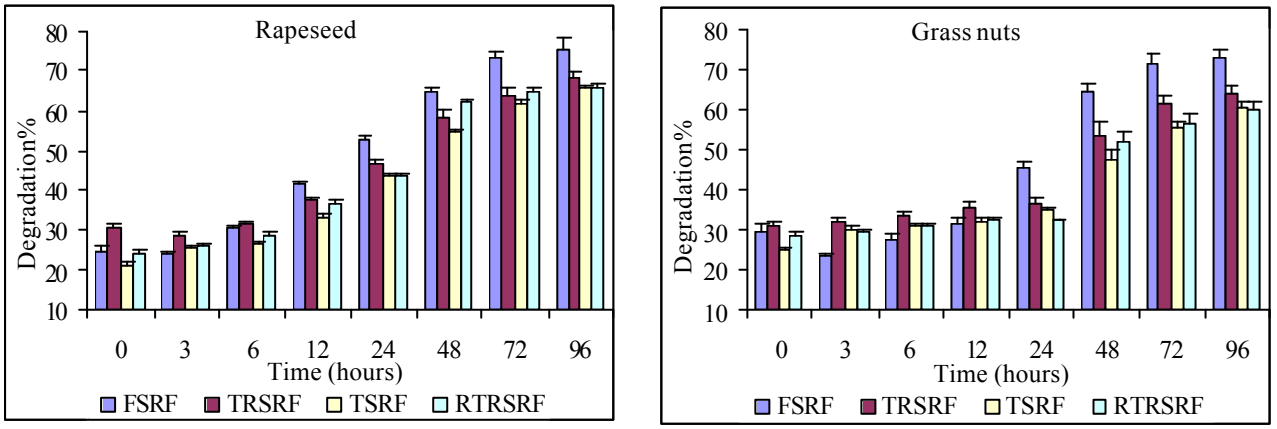
**Introduction** Fresh rumen contents are the most common source of inoculum for use to estimate in vitro degradation of ruminant feeds. However, the need to routinely access fistulated or slaughtered cattle to obtain rumen contents limits the availability of such inoculum and hence the applicability of in vitro methods by the commercial laboratories. Therefore, it would be advantageous, if rumen contents are preserved in sufficient quantity and used as a source for inoculum for use when there is a need to do so to estimate degradability of ruminat feeds. This study compared the suitability of frozen rumen contents as a source of inoculum to estimate in vitro rumen degradation of rapeseed meal (rapeseed) and grass nuts at various times.

**Material and methods** Whole rumen contents (WRC) were obtained from female cows, one freshly slaughtered on each of four separate occasions and were used to prepare four inocula as follows: WRC were divided into two parts. One part was immediately stored at –20°C for eight weeks (FWRC). The second part was strained through 4 layers of cheesecloth to obtain fresh strained rumen fluid (SRF) (FSRF) and the residual solids (RWRC) were stored at –20°C for two weeks. RWRC were thawed overnight at 39ºC in an airtight container and squeezed through cheesecloth to obtain thawed residual SRF (TRSRF). FWRC were also thawed overnight at 39ºC and squeezed through cheesecloth to obtain thawed SRF (TSRF), whereas the thawed residual FRWC (TRWRC) were stored at -20°C for further two weeks. TRWRC were then re-thawed to obtain re-thawed residual SRF (RTRSRF). Each inoculum was mixed with a buffer (ratio 1:4) and this buffered inoculum was kept at 39ºC until required in this experiment. The experiment was conducted as a 2×4×8×4 factorial design in duplicate and involved two feeds (rapeseed & grass nuts), four inocula (FSRF, TRSRF, TSRF and RTRSRF), eight incubation times, (0, 3, 6, 12, 24, 48, 72 and 96 h) and four cows as replicates. About 0.4g of rapeseed or grass nuts (<1mm) was weighed into a test tube to which 50ml of buffered inoculum were added, flushed with $Co_2$, capped with a Bunsen valve and incubated at 39ºC for various times. At the end of each incubation the residues were obtained after centrifugation, washed, dried and weighed to estimate disappearance of dry matter (DMD). The data were subjected to general linear model to compare the effect of various inocula on the mean DMD by using SPSS statistical package. Significance was declared if $P < 0.05$.

**Results** Mean (±s.e.) in vitro DMD of each feed at various times are shown in fig1.




As illustrated in Figure 1, the DMD increased with time. The DMD of both feeds for thawed inocula (TRSRF, TSRF and RTRSRF) were lower than their fresh counterparts (FSRF) at 24 hours onward. This may be because the microbial activity in thawed SRF was modified during thawing following storage at -20ºC. However, TRSRF showed slightly higher degradability values during first 6 hours than other inocula. Significant differences were found between means of different inocula ($P<0.01$). When averaged over all incubation times, the DMD (%) for FSRF, TRSRF, TSRF and RTRSRF were respectively 48, 46, 42 and 44 (s.e.m. 0.66) for rapeseed and 46, 44, 40 and 40 (s.e.m 0.93) for grass nuts. No significant difference was found between TSRF and RTRSRF for grass nuts although RTRSRF was exposed twice to freezing and thawing. For both feeds TRSRF tended to produce higher DMD values than TSRF. However, the overall DMD for rapeseed and grass nuts following in vitro incubation with the thawed inocula remained within the expected range of in vitro DMD for these feeds (Madsen and Hvelplund, 1985).

**Conclusion** The DMD values for thawed inocula were lower than their fresh counterpart for both feeds at most times. However, the overall degradation patterns of these feeds for thawed and fresh inocula were comparable. Thus, it may be possible to use frozen rumen contents as an alternative source to obtain inoculum when fistulated or slaughtered cattle are not available to supply fresh inoculum to rank ruminant feeds on the basis of their in vitro DMD. However, future studies should explore the reasons of lower DMD which may help us improve the procedures to obtain thawed inocula.

**Reference** Madsen, J. and Hvelplund, T. 1985. Protein degradation in the rumen. A comparison between in vivo, nylon bag, in vitro and buffer measurements. *Acta Agricultuae Scandinavica Supplement* **25:** 103-124

# The affect of rumen fluid collection time on its fermentative capacity and the stability of rumen conditions in sheep fed a constant diet.

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**Introduction:** One of the sources of variation in the *in vitro* gas production (GP) technique (Theodorou *et al.*, 1994) is the inoculum source that is used. The time of rumen fluid collection in relation to the feeding time of the donor animals could greatly affect the microbial activity and hence fermentative capacity of the inoculum. This study was carried out to quantify both within day and between day variation in rumen fermentative capacity.

**Materials and methods:** Six mature rumen canulated wethers were individually penned. All sheep were fed 460 g/d of a standard sheep concentrate. The hay component of the diet was either fed *ad libitum* as chopped hay or 900g/d as a component of a complete diet (restricted). The concentrate supplement and complete diet mix were fed to the animals in two equal portions twelve hours apart. Water was available *ad libitum.* Samples of rumen fluid were collected from each sheep for 32 consecutive days following a ten day adaptation period. Rumen fluid was collected prior to feeding from each sheep daily. In addition once a week (for the last 3 weeks of the experiment) rumen fluid was collected Pre-feeding, 4 and 8 hours post feeding. All samples of rumen fluid were analysed for their pH and VFA content. The weekly collections at pre-feeding, 4 & 8 h post feeding were also used to inoculate an *in vitro* fermentation of a standard starch substrate. The *in vitro* technique was based on the method described by Theodorou *et al.,* 1994. Modifications to the technique involved the use of 250 ml culture vessels, 200ml of diluted inoculant and 2 g of substrate. The experiment was analysed as a randomised block design.

**Results:** Variation in the capacity of the inoculum to ferment starch was found to be greatest for rumen fluid collected pre-feeding and least for samples collected 8 h post-feeding (Figures 1,2 & 3). Overall mean GP and rumen pH were found to be significantly affected by collection time. Mean PSI were found to be 42.3, 34.1, 37.4 (P=0.004, SED 2.57) and rumen pH 6.25, 5.96, 6.13 (P<0.001, SED 0.06) for inoculant collected pre-feeding, 4 & 8 h post feeding respectively. Day-to-day variation in rumen pH was found to be less variable when the sheep were fed the hay portion of the diet in a restricted manner (P<0.001 day.diet interaction SED = 0.11).

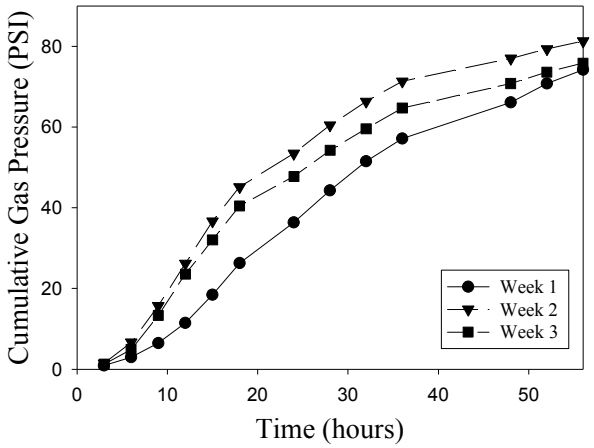


**Figure 1.** Pre-feeding.

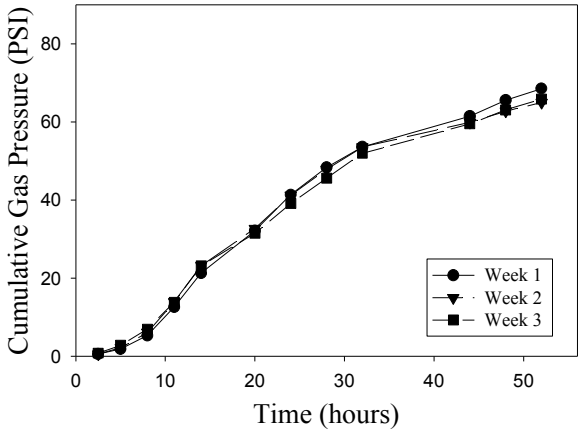


**Figure 2.** 4 h Post feeding

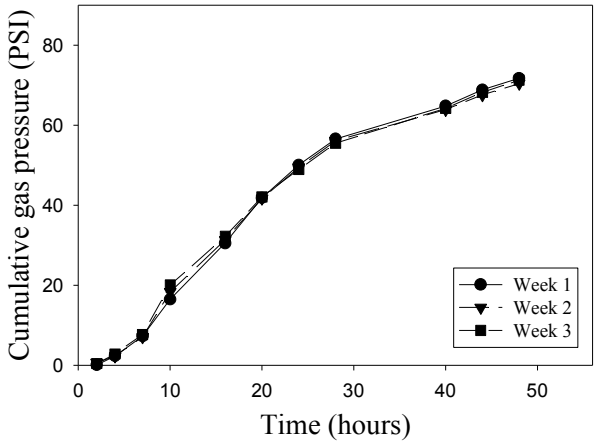


**Figure 3.** 8 h Post feeding

**Conclusions:**. Collection time of rumen fluid can have an affect on the overall and reproducibility of gas production profile of a standard substrate. Depressed gas production for the fermentation inoculated 4 h post-feeding is likely to have resulted from the depressed rumen pH. Day-to-day variation in rumen pH between sheep was found to be less when sheep were fed a restricted diet. In order to standardise the GP technique to facilitate comparisons between institutions the use of diets fed in a restricted manner with rumen fluid collected 4 or 8 h post feeding is recommended.

**References**

Theodorou, M.J., Williams, B.A., Dhanoa, M.S., McAllan, A.B. and France, J. 1994. A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. *Animal feed science and technology.* **48** 185-197.

# Variation in the *in vitro* hydrolytic activity of rumen and faecal inocula

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**Introduction** Considerable efforts have been made regarding the use of faecal material to provide a microbial inoculum for *in vitro* feed evaluation systems. However total gas production, rate of gas release and the extent of degradation of feeds incubated using faecal inoculum are lower than those incubated in a rumen fluid medium. It has been suggested that this is due to lower microbial activity, a consequence of the different microflora and reduced microbial numbers (e.g. Mauricio, 1999). Microbial populations are dynamic so, as their enzyme activity profiles change rapidly, little information is obtained from examining these. However, their hydrolytic activity as reflected by their ability to degrade specific substrates can be simply measured and provides a potential method with which to assess the quality of inocula with respect to their use in *in vitro* systems. The data presented here are from a larger study in which the differences between the hydrolytic activity of faecal material and rumen contents as influenced by the time of sampling were assessed *in vitro* .

**Materials and methods** Rumen fluid (hand squeezed contents) and faecal samples (*per rectum*) were obtained one hour before feeding at 07.00 or 15.30 h, from two dry cows offered a diet of hay and grass silage, on two occasions. The faecal material was blended (30 seconds) with an equivalent volume of reduced buffer and strained twice through a single cloth. The rumen fluid was strained through a double layer of cloth and both inocula held under $CO_2$ at 39 °C until used (<1 h after sampling). The sacchrolytic, amylolytic and fibrolytic activity of the inocula were examined by incubating xylose (*X*), starch (*S*) and cellulose (*C*), respectively over a 48 h period at 39 °C using the Reading Pressure Technique An estimate of cell wall degradation kinetics was provided by a fourth substrate, washed hay (*WH*). Head-space gas pressure readings were taken 2, 4, 6, 8, 10, 12, 15, 19, 24, 30, 36 and 48 h post-inoculation and three replicates per substrate plus negative controls were withdrawn at 6, 12, 19, 24 and 48 h to determine degradability. SAS GLM procedures were used to generate LS means and significances of difference.

**Results** The extent of *WH* degradation was significantly reduced when the faecal, compared to rumen fluid, was used, (Table 1). Although there was a tendency for the pm rumen inoculum to show a reduced rate of degradation up to 24 h this effect was not significant. Both inocula ranked the four test substrates in a similar order with respect to cumulative gas production ($S > X > C > WH$), however the 48 h gas release values for the faecal inocula were significantly lower and generally equal to the 24 h values obtained with the rumen fluid (Table 2). Not only were lag times extended when faeces were used, but unlike rumen fluid, the least fermentable substrates (*C* and *WH*) could not be differentiated even at 48 h. As expected little difference between sampling time, was identified with respect to activity of the faecal material. In contrast rumen samples obtained at 15.30 h showed significantly lower rates of gas release than those taken at 07.00 h. Due to similarities between the *S* fermentation curves it can be concluded that the amylolytic activity of rumen fluid and faeces were, in this study, equivalent. However marked differences in the rate of gas release when *X* and to a lesser extent *C* were incubated suggest compositional differences in the rumen and faecal microflora. This lends support to the idea that the faecal microflora tends to be facultative characterised by fewer, opportunistic microorganisms, while the rumen microflora is highly specialised, exhibiting considerable diurnal variation due to substrate availability.

**Table 1** Influence of inoculum and sampling time on washed hay degradation (g $kg^{-1}$ OM)

| Inoculum | Time (h) | Degradation g $kg^{-1}$ 12 h | 24 h | 48 h |
|---|---|---|---|---|
| Rumen | 07.00 | 39a | 299a | 523a |
| | 15.30 | 35ab | 241a | 521a |
| Faeces | 07.00 | 15b | 62b | 407b |
| | 15.30 | 23b | 82b | 399b |
| se means | - | 2.7 | 8.1 | 5.8 |

**Table 2**: Cumulative gas release by substrate as influenced by inoculum

| Inoculum | Substrate | Cumulative gas release (ml g $OM^{-1}$) 6h | 12h | 19h | 24h | 48h |
|---|---|---|---|---|---|---|
| Rumen | Cellulose | 5.0d | 12.4e | 44.8d | 92.6d | 260.7bc |
| | Starch | 78.1c | 230.0a | 297.9a | 323.5a | 356.8a |
| | Xylose | 24.6d | 101.1c | 216.0b | 255.6b | 294.0b |
| Faeces | Cellulose | 1.2d | 3.4e | 6.3e | 16.1e | 114.0a |
| | Starch | 34.2a | 202.3b | 278.5a | 298.3a | 325.1ab |
| | Xylose | 3.5b | 59.4d | 161.8c | 188.3c | 233.3c |
| se means | - | 0.90 | 2.87 | 3.51 | 4.28 | 6.49 |

LS Means in columns without similar letters are significantly different (P<0.05)

**Conclusions** The study has confirmed the lower rates of fermentation and degradation associated with the use of faecal material as an inoculum for *in vitro* feed evaluation. A significant effect of sampling time on the quality of the inoculum with respect to rumen fluid but not faeces, was identified. The identified variation in hydrolytic activity suggests compositional differences in the microflora, indicating that care should be exercised when extrapolating results, obtained *in vitro* using faecal inocula, to the practical situation.

**References**

Mauricio, R.M. 1999. *Comparison of bovine rumen liquor and bovine faeces as sources of microorganisms for an in vitro gas production technique for evaluating forages*. PhD thesis, University of Reading, Reading, U.K.

# Variation in gas release profiles *in vitro* as influenced by volatile fatty acid composition and rate of addition to two standard incubation media

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**Introduction** *In vitro* gas systems characterise substrate degradation by estimating fermentation gas release over time. However, as this offers little information regarding feedstuff utilisation, the extent of organic matter degradation (OMD) must be measured to provide an estimate of the metabolizable end-products (VFA and microbial protein) produced. Systems where capacity is limited use either an incubation end-point derived partitioning factor (PF) to estimate degradation from gas or attempt to model OMD stoichiometrically from VFA analyses or assumed gas / VFA relationships. These estimates contain numerous errors. For instance the PF is not constant and the stoichiometric calculations used assume that all OM degraded is carbohydrate composed of hexose sugars. A further potential error is the estimate of the proportion of released gas that originates from VFA neutralisation, as this varies with buffer composition and both proportion and rate (i.e. concentration) of VFA production.

**Materials and methods** Three mixtures of acetic:propionic:butyric acids were prepared (0.68:0.23:0.09, 0.53:0.35:0.12 and 0.42:0.42:0.16) to represent rumen VFA compositions of hay:concentrate diets with 1.0, 0.4 and 0.2 hay, respectively. These were diluted to provide three final concentrations (45, 60 and 75 μm mol $l^{-1}$), typical of those found in the rumen, to be achieved. Two buffers (Mauricio *et al*., 1999 and Beuvinc and Spoelstra, 1982) referred to as A and B, respectively were used. They differed in their initial pH (8.12 and 7.74) and the quantity of phosphate and carbonate (3.26 and 8.11, 2.98 and 9.75 g $l^{-1}$) for buffers A and B, respectively. These were prepared using only the macromineral and buffer components and were neither reduced nor gassed with $CO_2$. Sealed fermentation flasks containing 100 ml buffer and fitted with two gas-tight ports, were used to estimate gas release. The first port was to permit addition of acid in 0.1 ml aliquots using a graduated syringe, while the second allowed head-space gas pressure to be measured and the flask vented. Following acid addition the flask was shaken and gas pressure measured after 30 minutes. The flask was then vented and the process repeated over a nine hour period. Five replicates were used per VFA mixture*concentration*buffer interaction, three for gas estimation and two to examine changes in pH. Gas pressures were transformed to volume and SAS GLM procedures used to generate LS means and significances of difference.

**Results** A slight but significant difference was observed between the two buffers, with A producing about 1.0 ml less gas than B and showing a greater decline in pH. As this buffer contained less $CO_3^{2-}$ and more $PO_4^{3-}$ than B, it is likely that more acid was neutralised by $PO_4^{3-}$ resulting in less $CO_2$ being released. Although the pH of both buffers declined steadily, end-point values were not sufficiently low to impair fermentation. As expected gas production was related to the quantity of acid added, although this relationship was curvilinear within the range examined, with the rate of gas release increasing with increasing levels of acid addition. The significant decline in pH was a direct function of quantity of acid addition. Of marked interest was the observation that gas release varied significantly with the VFA mixture added and was greatest with the high propionic acid mix. End-point buffer pH for this mixture also showed the largest reduction. This indicates that pK of the VFA mix, in addition to concentration, determines gas release.

**Table 1**: Gas release and pH as influenced by buffer, VFA proportion and concentration.

| Factor | Level | Gas release (ml) | | | Buffer medium pH | | |
|---|---|---|---|---|---|---|---|
| | | 3 h | 6 h | 9 h | 3 h | 6 h | 9 h |
| Buffer | A | 9.29b | 24.23b | 41.10a | 7.16b | 6.81b | 6.56b |
| | B | 9.86a | 25.24a | 42.15a | 7.26a | 6.96a | 6.78a |
| VFA mix | 1 | 9.88a | 24.60b | 41.08b | 7.40a | 7.11a | 6.94a |
| | 2 | 9.66ab | 23.98b | 40.67b | 7.15b | 6.85b | 6.66b |
| | 3 | 9.18b | 25.63a | 43.13a | 7.08c | 6.69c | 6.41c |
| Concentration (μmol $l^{-1}$) | 45 | 8.79b | 20.67c | 33.12c | 7.30a | 6.93a | 6.66b |
| | 60 | 8.59b | 22.98b | 39.58b | 7.16b | 6.88b | 6.73a |
| | 75 | 11.34a | 30.55a | 52.18a | 7.18b | 6.84b | 6.63b |
| s.d. | | 0.688 | 1.325 | 1.909 | 0.053 | 0.042 | 0.044 |

Means in columns within factor without similar letters are significantly different (P>0.05)

**Conclusions** These results suggest that the composition and concentration of VFA produced, together with the type of buffer used, have a significant effect on the quantity of gas released from neutralisation of fermentation acids. This finding has a major impact on the ability of models that estimate substrate degradability from cumulative gas profiles.

**References**

Mauricio, R.M., Mould, F.L., Dhanoa, M.S., Owen, E., Channa, K.S. and Theodorou, M.K. 1999. A semi-automated in vitro gas production technique for ruminant feedstuff evaluation. *Animal Feed Science and Technology* **79**: 312-330.
Beuvinc, J.M.W. and Spoelstra, S.F. 1992. Interactions between substrates, fermentation end-products, buffering systems and gas production upon fermentation of different carbohydrates by mixed microorganisms in vitro. *Applied Microbiology and Biotechnology* **37**: 505-509.

# The rumen degradability of crimped wheat in comparison with conventionally treated grain assessed *in vitro*

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**Introduction** Despite little published research data (Ekström *et al*., 1966) the use of crimped cereal grain in the rations of high producing dairy cows has gained considerable acceptance. This is partly due to an earlier harvesting date permitting replanting up to three weeks earlier and to reduced storage costs. However, the main attraction is that high levels of crimped grain are readily consumed without apparently adversely affecting rumen fermentation. Although this appears to be in direct contradiction with the belief that crimped grain is fermented faster due the ensiling process, this information was probably obtained using the *in sacco* technique which tends to over-estimate processed cereal degradation due of fine particulate losses (e.g. starch granules). This study was therefore undertaken to characterise the degradation profile of crimped wheat grain relative to that of conventionally harvested material.

**Materials and methods** A sample of conventionally harvested and stored wheat was either left whole (*W*) or roll-milled (coarse, *C*; medium, *M*; and fine, *F*). Crimped wheat (*E*) was prepared from the same source using grain harvested at approximately 600 g DM $kg^{-1}$, lightly rolled and ensiled. Degradation and fermentation profiles were generated using the Reading Pressure Technique (Mauricio *et al*., 1999). In a single study, three replicates of each substrate were used for each of the seven withdrawal periods (6, 12, 19, 24, 48, 72 and 96 h) to determine dry and organic matter degradation (DMD and OMD, respectively) from recovered fermentation residues and head-space gas pressure measurements at 2, 4, 6, 8, 10, 12, 15, 19, 24, 30, 36, 48, 72 and 96 h post-inoculation. The buffered rumen fluid inoculum was prepared from hand-squeezed rumen samples obtained pre-feeding (07.00h) from a cow offered a high concentrate (550 g $kg^{-1}$) ration. The data were analysed using SAS GLM procedures to obtain LS means and significances of difference.

**Results** Whole wheat released the lowest quantity of gas over the incubation period (50 ml). In contrast *E* was highly fermentable producing 110 ml gas by 6 h post-inoculation and over 360 ml at 96 h. Particle size reduction improved accessibility to starch and significantly increased gas production from 6 h to 48 h. This difference was still apparent at 96 h. Not only did processing (rolling) increase the rate of gas release (*F*>*M*>*C*, Figure 1) but peak rate occurred earlier. However *E* not only produced the highest peak yield (25 ml $h^{-1}$) but this occurred earliest (6 h post-inoculation). As ash contents varied only slightly, DMD and OMD profiles were similar. OMD of *W* was poor (180 g $kg^{-1}$ at 96 h), reflecting the intact seed coat, in comparison with the other conventional substrates all of which were degraded in excess of 950 g $kg^{-1}$. In contrast and throughout the incubation period although initial degradation was high (424 g $kg^{-1}$ at 6 h) *E* was degraded to a significantly lower extent (Table 1). The large soluble component of *E* results from acid hydrolysis of starch and conversion to acid during the ensiling process, while the poorer subsequent degradation suggests that this material contains a greater proportion of fibrous material.

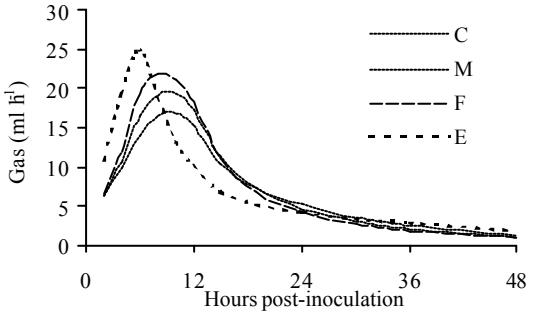


**Figure 1** Influence of substrate on rate of gas release

**Table 1** Organic matter degradation (g $kg^{-1}$)

| Substrate | Hours post inoculation | | | | |
|---|---|---|---|---|---|
| | 6 | 12 | 24 | 48 | 96 |
| W | 17d | 31e | 63d | 122c | 180c |
| C | 346c | 586c | 768b | 922a | 954a |
| M | 420b | 653b | 854a | 941a | 961a |
| F | 513a | 701a | 881a | 945a | 954a |
| E | 424b | 525d | 670c | 813b | 928b |
| se means | 2.5 | 2.9 | 4.5 | 2.7 | 2.3 |

Means in columns without common letters are significantly different (P>0.05)

**Conclusion**. It is therefore concluded that, although *E* has about 300 g $kg^{-1}$ less organic matter than conventional grain on a fresh weight basis, more *E* can be consumed without adversely affecting rumen fermentation for a number of reasons. Less readily fermentable material is present as a portion of the starch present at harvest has been converted to acid during the ensiling process. In turn these acids cannot be fermented further in the rumen. Finally the actual organic matter is less degradable as it contains a higher fibre (seed coat) component. These data indicate that the degradation profiles of crimped and conventional grains differ to such an extent that they can be considered as essentially different substrates rather than the same substrate subjected to different degrees of processing. It is also identified that an economic assessment of crimped versus conventionally harvested grain would provide much needed information however this would requires that an accurate utilisable metabolisable energy inventory to be generated.

**References**

Ekström, N, Thomle, S. and Schmekel. J. 1966. Utfodring av spannmålsensilage [Feeding ensiled grain]. *Swedish Institute of Agricultural Engineering, Report no. 316*, Uppsala, Sweden.
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# Impact of feed supplements on *in vitro* degradability of barley straw and grass nuts

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**Introduction** Grass is the main energy feed for cattle but it declines in digestibility and intake during the summer months and loses about 20% nutrients during ensiling and feeding in winter. Grass is also low in minerals and this has implications for cattle health and performance. It is thus essential to use supplements to boost digestibility and intake of grass and subsequently the performance of cattle. This study compared the effect of two supplements which are marketed as molasses based feed blocks (Booster with 12% crude protein or CP and HIPRO with 28%: CRYSTALYX) on the *in vitro* dry matter (DM) degradation of barley straw (Straw) and grass nuts (Grass). These blocks contained same energy (13MJ ME/kg DM) but different amounts of sugars (33-35%), oil (6-8%) and minerals (20 to 28%) and thus were considered appropriate for use as feed supplements to compensate for the deficits of nutrients that the cattle can face when consuming grass or straw. These forages represent a range of forage quality that can be found in the UK.

**Materials and Methods** The study was conducted according to a 2 x 2 x 3 x 6 factorial design, with three replicates and involved 2 forages (Straw and Grass), 2 CRYSTALYX (Booster and HIPRO) each with 3 levels (0, 9 and 18g /100g forage) and 6 incubation times (0, 6, 12, 24, 48, 72 hours). Each replicate was run on a separate occasion by using strained rumen fluid (SRF) from different cattle slaughtered freshly. One portion of SRF was mixed with 4 portions of a buffer and was used as an inoculum. About 1g ground (<1mm) straw or grass was weighed separately into a test tube to which relevant amount of Booster or HIPRO and 50ml of inoculum were added. The tubes were then flushed with $CO_2$, capped with Bunsen valves and incubated at 39$^{o}$C for various hours. The undegraded residue was then collected from each tube, washed with water, dried at 70$^{o}$C and analysed to estimate *in vitro* DM disappearance (DMD) from both forages in response to CRYSTALYX inclusion at each time. The data were then fitted exponentially to derive degradation constants (*a, b and c*) and predicted degradability ($P_{0.02}$). The data on DMD, degradation constants and $P_{0.02}$ were then statistically analysed by using PROC GLM in SAS. The model studied the main effects of forage and the type (Type) and amount (Amount) of CRYSTALYX and declared the effect significant if $P<0.05$.

**Results** Table 1 presents mean (per *3 cows) in vitro* DMD (g /kg) of grass nuts and barley straw in response to various levels (0, 9 and 18g /100g forage) of Booster or HIPRO during different hours of incubations with buffered rumen fluid.

| **Forages** | **Grass nuts** | | | | | | | | **Barley straw** | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Supplement** | **Booster %** | | | | **HIPRO %** | | | | **Booster %** | | | | **HIPRO %** | | | |
| Incubation hours | **0** | **9** | **18** | ***SE*** | **0** | **9** | **18** | ***SE*** | **0** | **9** | **18** | ***SE*** | **0** | **9** | **18** | ***SE*** |
| 0 | 229 | 273 | 310 | *24* | 236 | 278 | 258 | *12* | 106 | 159 | 224 | *34* | 21 | 81 | 146 | *36* |
| 6 | 263 | 242 | 257 | *6* | 243 | 292 | 282 | *15* | 95 | 148 | 200 | *31* | 85 | 140 | 168 | *24* |
| 12 | 271 | 270 | 321 | *17* | 277 | 317 | 362 | *25* | 104 | 154 | 183 | *23* | 116 | 164 | 171 | *17* |
| 24 | 358 | 374 | 388 | *9* | 327 | 398 | 390 | *23* | 129 | 174 | 198 | *20* | 113 | 167 | 177 | *20* |
| 48 | 450 | 517 | 523 | *24* | 519 | 543 | 539 | *8* | 223 | 309 | 231 | *28* | 243 | 254 | 297 | *17* |
| 72 | 585 | 569 | 567 | *6* | 609 | 625 | 620 | 5 | 277 | 321 | 349 | *21* | 310 | 353 | 389 | *23* |
| ***SE*** | *56* | *57* | *51* | | *64* | *59* | *58* | | *31* | *33* | *25* | | *44* | *39* | *40* | |

The DMD of Grass and Straw were improved in response to either Booster or HIPRO addition at most times. As expected, Grass showed higher DMD than Straw at each corresponding time. However, Straw showed more consistent increase in DMD in response to increasing amounts of Booster or HIPRO at most times. When compared with 0% or no CRYSTALYX addition (Control), the low level of CRYSTALYX addition (9%) showed a 15% increase in DMD whereas high level of CRYSTALYX addition (18%) showed a 22% increase in DMD. The main effect of Forage was significant ($P<0.001$) for DMD, degradation constants (not *'c'*) and $P_{0.02}$. However, the main effects of Type and Amount of CRYSTALYX were significant ($P<0.01$) for only *a'* and DMD respectively. Other main effects or their interactions were not significant ($P>0.05$). It appeared that Grass was more soluble (*a*) and potentially more degradable (*b*) with a faster degradation rate (*c*) and consequently showed greater predicted degradability ($P_{0.02}$) than Straw. Booster was more soluble but slow in degradation of potentially degradable fraction and consequently showed lower predicted degradability than HIPRO. The predicted degradability showed an increase of 17% when the low level of CRYSTALYX addition was compared with that where no CRYSTALYX was added.

**Conclusion** Both supplements were able to improve *in vitro* digestion of barley straw and grass nuts. The increased digestion was perhaps a result of enhanced rumen microbial fermentation in the presence of supplements containing nutrients (sugar, protein and minerals) lacking in these forages. It is suggested that enhanced rumen fermentation could stimulate forage intake and hence the performance of cattle. Therefore, animal studies are being conducted at the University of Newcastle to explore the benefits of feeding supplements to grass fed ruminant livestock in terms of their performance under various feeding and production situations.

***Acknowledgments*** to Caltech for part funding and Mr. Mike Hearn for his assistance during laboratory analysis.

# An *in vitro* model to assess the impact of lipid on the rate and extent of fibre degradation

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**Introduction** Although high genetic merit dairy cows are capable of peak yields in excess of 50 kg per day these are seldom maintained primarily due to an inability to satisfy energy requirements. Fats and oils are often incorporated into rations as a means of increasing dietary energy content. However, unless included at relatively low levels or in a protected form, lipid supplements may adversely affect fibre degradation in the rumen. The current data are derived from a study conducted to evaluate the potential of an *in vitro* model to assess the effects of oil supplements on forage organic matter degradation (iOMD).

**Material and methods** Three commercial cold-pressed lipid supplements, cod liver (*F*), linseed (*L*) and rapeseed (*R*) oil, were assessed at four inclusion levels: 20, 40, 80 and 120 mg $g^{-1}$ grass hay (947 g $kg^{-1}$ DM) according to a randomised factorial design. The Reading Pressure Technique (Mauricio *et al*., 1999) was used to estimate the rate and extent of gas release and iOMD during a 48 h incubation period. Three replicates of each substrate were used for each of five withdrawal periods (6, 12, 18, 24 and 48 h post-inoculation). Untreated hay samples (*H*) were also examined, in addition to controls where lipids were directly added to the buffered rumen fluid incubation medium. The inoculum was prepared from hand-squeezed rumen samples obtained pre-feeding (07.00h) from a lactating dairy cow offered a hay-grass silage based diet. Cumulative gas release profiles were generated from head-space pressure values obtained at 2, 4, 6, 8, 10, 12, 15, 18, 24, 30, 36 and 48 h post-inoculation. The GLM procedure of SAS was used for statistical evaluation of experimental data.

**Results** The rate of gas release profile of the control hay (H) clearly identified the fermentation of cell contents (4 h), primary (12h) and secondary (18 h) cell wall components. Oil supplements increased the rate of gas release 6 to 8 hours post-incubation, possibly due to fermentation of glycerol liberated during lipolysis (Figure 1). Thereafter, the rate of gas release relative to H was reduced by lipid supplementation. Consistent with the rate of gas release, inclusion of oil supplements also influenced iOMD, the extent of which was dependent on both oil type and level of inclusion

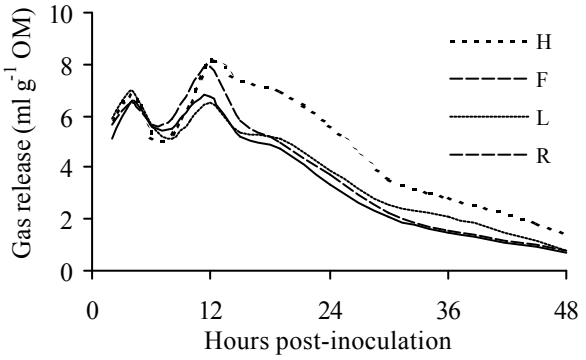


**Figure 1** Mean effect of lipid supplements on the rate of gas release

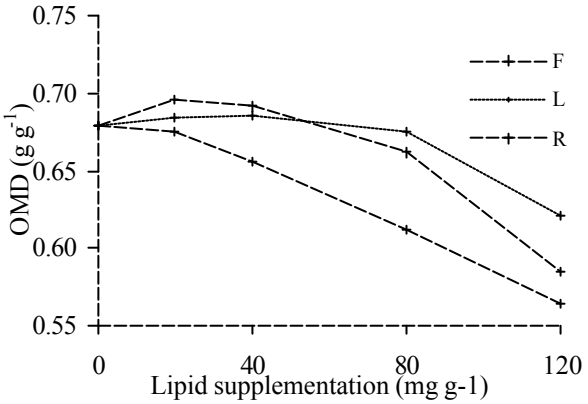


**Figure 2** Effect of lipid supplements on iOMD

(Figure 2). At low levels *R* improved iOMD, but at inclusion levels above 40 mg $g^{-1}$ iOMD was progressively decreased. *L* had only minor effects on iOMD up to 80 mg $g^{-1}$, but at levels above 120 mg $g^{-1}$ *L* and *R* significantly depressed iOMD. In contrast, *F* significantly reduced iOMD at levels >20 mg $g^{-1}$, the extent of which was linearly dependent on the level of supplementation. The similarity in the gas release curves up to 10 h post-inoculation suggests that iOMD reduction was associated with a depression of cell wall degradation, particularly that of secondary cell wall components.

**Conclusion** The current data demonstrated that the *in vitro* model was sufficiently sensitive to discern differences between lipid supplements, with respect to forage cell wall degradation, indicating the potential of this system to assess the efficacy of techniques that render lipid supplements inert in the rumen. This suggests the method has potential in establishing the degree of rumen protection required for the modification of the fatty acid composition of ruminant products (milk and meat), using dietary lipid supplements. Furthermore, development of this approach could allow detailed examination of fatty acid biohydrogenation pathways, and thereby identify conditions necessary for enhancing the synthesis of trans-vaccenic acid and conjugated dienes of linoleic acid in the rumen.

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# Chemical composition, digestibility and predicted energy value of whole-crop forage lupins

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**Introduction** The seeds from newer, varieties of determinate lupin grown in the UK are potentially a useful source of protein to livestock. Also, their ability to be cultivated in a temperate climate may afford the opportunity to reduce the dependence on imported protein sources such as soya bean meal. There is also currently interest in assessing the potential for harvesting whole-crop forage lupin (WCFL) as a high-protein forage crop for ruminant animals. The aim of the present study was to examine the nutritional value of WCFL samples harvested at different stages of maturity.

**Materials and methods** Two crops (Site 1: Oxon; Site 2: Hampshire) of white, winter indeterminate lupin, variety Athos, were sown in 4 ha sites at a rate of 125 kg seed $ha^{-1}$ on 06.10.97. Five samples (S1-5) were harvested during 1998 (Site 1: S1, S2, S4 and S5 on 15.06, 15.07, 09.08 and 18.08 respectively; Site 2: S3 on 03.08). Also, a further sample of S2 was fractionated into five plant parts; lower stem to first seed pods (F1), stem between first and second seed pods (F2), all remaining stem (F3), leaves (F4) and first and second set of seed pods (F5). Each sample, including F1-5, was dried at 100°C and 70°C for 1 and 17 h respectively, and then oven dry matter (ODM) content calculated. The samples were then milled (1 mm screen) and analysed for total ash, crude protein (CP; nitrogen x 6.25) and *in vitro* organic matter digestibility (*invitro* DOMD) using the method of Tilley and Terry (1963). Also, samples S1-5 were analysed for water soluble carbohydrates (WSC), neutral detergent fibre (NDF) and neutral detergent-cellulase plus gamannase digestibility (NCGD).

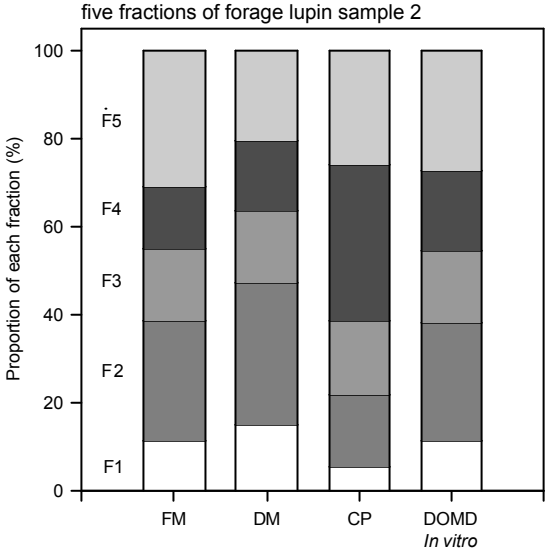
**Results** The results (Table 1) show an increase in ODM and NDF content, and a reduction in digestibility and predicted ME content with increasing maturity. The ODM of S1 and S2, taken from the standing crop, was very low. ODM was increased following 3 d wilting or desiccation using Roundup for 5 or 18 d for S3, S4 and S5 respectively. There was little change in overall CP content with increasing maturity (range 144 to 173 g $kg^{-1}$ DM). For S2 (Figure 1) F4 and F5 accounted for 61% of the total CP (35 and 26% for F4 and F5 respectively).

Table 1. Chemical composition, *in vitro* digestibility and predicted energy content of whole-crop forage lupin samples (as g $kg^{-1}$ DM unless stated).

| Det[n] | Sample identification | | | | |
|---|---|---|---|---|---|
| | S1 | S2 | S3 | S4 | S5 |
| ODM[1] | 128 | 160 | 239 | 184 | 322 |
| Ash | 76 | 59 | 40 | 60 | 64 |
| WSC | 120 | 118 | 62 | 48 | 28 |
| CP | 173 | 150 | 158 | 144 | 154 |
| NDF | 488 | 517 | 573 | 589 | 595 |
| NCGD | 669 | 625 | 610 | 583 | 558 |
| *invitro* DOMD | 621 | 600 | 564 | 586 | 527 |
| ME[2] | 9.4 | 8.8 | 8.5 | 8.1 | 7.8 |

[1] ODM as g $kg^{-1}$
[2] predicted from the equation (ADAS, 1993):
ME (MJ $kg^{-1}$ DM) = -0.61 + 0.0150*NCGD

Figure 1. Distribution of total fresh and dry matter, crude protein and digestible organic matter between five fractions of forage lupin sample 2




**Conclusions** The standing lupin crop was of very low ODM (mean of S1 and S2, 144 g $kg^{-1}$) and will require in-field treatment (wilting or desiccation) in order to increase overall ODM content prior to ensilage. The low WSC content at ensilage suggests an additive will be required at ensilage. While the energy content decreased with increasing maturity, the WCFL had a high CP content which remained relatively constant throughout the growing period studied. While the leaves (F4) comprised the greater proportion of the total CP content of S2 further work is required to establish the changes which occur in the distribution of CP throughout the crop with increasing maturity. Work to identify the most suitable genotypes of lupins for WCFL should also be carried out.

**Acknowledgements** We wish to acknowledge the partial funding of this work from the Maize Growers Association.

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# Prediction of ADF and NDF in faeces by NIRS to assess diet composition in grazing animals

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**Introduction** Chemical analysis have been useful in characterising both nutrient content and digestibility of forages but less useful in predicting voluntary intake by animals (Ward et al., 1982). Faeces is the product of eroding and synthesising digestive processes and consists of residues of feed and plant tissue, component of microbial and animal origin, for this reasons faeces should contain information about the amount and characteristics of the diet. Near infrared reflectance spectroscopy (NIRS) is widely used to predict quality characteristics in forages and several reports (Lyons and Stuth, 1992; Leite and Stuth, 1995, Coates, 1999) indicated that useful prediction of dietary digestibility in grazing ruminants using faecal NIRS analysis. It is assumed for different authors that rangeland herbivore faeces contains chemical bonds resulting from undigested residues and microbial fermentation and host animal digestion end products which can provide NIRS spectral information highly correlated with dietary crude protein and digestibility (Lyons and Stuth, 1992). The objective of this work was to develop NIRS equation calibrations to estimate acid detergent fibre (ADF), neutral detergent fibre (NDF) and nitrogen in faecal samples to be used as a tool to estimate diet composition in ruminant animals under grazing conditions.

**Materials and Methods** 150 faecal samples from two fields experiments using beef cattle and dairy cattle animals, respectively, were used. In both experiments total collection of faeces was done. Samples were dry in an air force oven (65 $^0$C during 48 hours) and milled through a 1 mm screen using a Wiley mill (Arthur H. Thomas, USA). Nitrogen (N) was determined according AOAC (1990). Acid detergent fibre (ADF) and neutral detergent fibre (NDF) according to Goering and Van Soest procedures (1967). Samples were scanned dry on a NIRS 6500 spectrometer (NIRSystems, USA), using the small ring cup cell. Spectral data were recorded as log 1/Reflectance. NIRS calibration models were developed using two mathematical treatments to the spectra. D1: 1,4,4,1 and D2: 2,5,5,2. Standard normal variate and detrend (SNV-D) was applied to both mathematical treatments as scatter correction. The coefficient of multidetermination in calibration ($R^2_{CAL}$) and the standard error in calibration (SEC) were calculated.

**Results and Discussion** Table 1 presents the NIRS calibration statistics for the chemical parameters in faeces samples. Good NIRS calibration statistics were obtained for ADF and NDF respectively. Nitrogen was poor predicted by the NIRS calibration models.

**Table 1. NIRS calibration statistics for faecal samples (g kg $^{-1}$ DM).**

| | n | Mean | Range | SD | $R^2_{CAL}$ | SEC |
|---|---|---|---|---|---|---|
| N | | | | | | |
| *D1* | 110 | 17.9 | 13.5 – 21.6 | 1.5 | 0.78 | 0.07 |
| *D2* | 110 | 17.9 | 13.5 – 21.6 | 1.6 | 0.83 | 0.06 |
| ADF | | | | | | |
| *D1* | 120 | 449.5 | 275.1 – 505.2 | 41.3 | 0.91 | 1.2 |
| *D2* | 120 | 449.1 | 275.1 – 505.2 | 41.2 | 0.89 | 1.3 |
| NDF | | | | | | |
| *D1* | 116 | 486.1 | 373.9 – 553.9 | 35.7 | 0.86 | 1.3 |
| *D2* | 112 | 487.4 | 373.9 – 553.9 | 35.6 | 0.86 | 1.3 |

**Conclusions** NIRS shows the capability to be used as a tool to estimate chemical parameters in fecal samples. Further work will be done to correlate NIRS chemical parameters with intake under grazing conditions.

**Acknowledgements**
The work was supported by The National Institute for Agricultural Research – Uruguay (INIA)

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# Relationship between calpastatin activity and the slow and fast myosin heavy chain content of ovine skeletal muscles

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**Introduction** Calpastatin, the specific endogenous inhibitor of the calpain system, is considered to be a principle contributor to variations in meat tenderisation (Parr et al., 1999). Previous studies have suggested that the differences in calpastatin activity in different ovine skeletal muscles could be influenced by muscle metabolic and contractile characteristics according to myofibrillar ATPase activity (Ouali and Talmant, 1990). The type of myofibrillar ATPase activity is largely determined by the content of slow or fast myosin heavy chains (Rivero et al., 1999). The present study was designed to investigate the relationship between calpastatin inhibitory activity and slow myosin heavy chain (MHC-s) and fast myosin heavy chain (MHC-f) expression.

**Materials and Methods** Six 12 month old Mule x Charolais rams were slaughtered by electrical stunning and severance of the carotid arteries. *Longissimus dorsi* (LD), *tensor fasciae latae* (TFL), *semitendinosus* (ST), *trapezius* (TZ) and *supraspinatus* (SS) muscle samples were removed from the left side of each carcass within 5 min postmortem and immediately frozen in liquid nitrogen for subsequent calpastatin activity measurements and immunochemical fibre type analysis. Calpastatin activity was measured fluorimetrically (Sensky et al., 1996) in the supernatants of boiled muscle extracts, prepared from 1g of tissue homogenised in 100 mM Tris HCl buffer, pH 8.3 containing 10 mM EDTA, 2 mM dithiothreitol and 0.5 mM 2-(4-aminoethyl)-benzenesulphonyl fluoride. Whole muscle from the same samples was electrophoresed, blotted and immunodetected using MHC-s (1:1000) and MHC-f (1:1000) monoclonal antibodies (Novacastra). Blots were reprobed with rabbit anti-actin antibody (1:500, Sigma) to correct for protein load. Bands were visualised by CDP-star (Amersham) and quantified by Quantity One image analysis software (BioRad). Differences in calpastatin activity and fibre distribution between muscles were analysed by one-way analysis of variance. Regression analysis was used to correlate calpastatin inhibitory activity with MHC-s and MHC-f expression.

**Results** There were significant differences in calpastatin activity between the different muscles ($p < 0.001$) with the greatest and smallest calpastatin inhibitory activities observed in TZ and LD, respectively. The expression of MHC-s and MHC-f content also differed significantly between muscle types ($p < 0.001$ and $p < 0.05$, respectively) with TZ having the greatest MHC-s content and TFL having the greatest MHC-f content (Table 1). Significant positive and negative correlations were observed between MHC-s and calpastatin activity ($r^2 = 0.38$, $p < 0.001$) and between MHC-f and calpastatin activity ($r^2 = 0.45$, $p < 0.001$), respectively (Figure 1).

**Table 1** Effect of muscle group on fibre type distribution (OD/unit tissue) and calpastatin activity ($10^9$ fluorescence units/kg)

| Muscle | MHC-s | MHC-f | calpastatin |
|---|---|---|---|
| LD | 1.3 | 2.01 | 1.38 |
| TFL | 0.42 | 2.02 | 1.41 |
| ST | 1.52 | 1.79 | 1.93 |
| TZ | 4.62 | 1.3 | 2.93 |
| SS | 4.06 | 0.79 | 2.75 |
| sed | 0.73 | 0.37 | 0.33 |
| p | < 0.001 | < 0.05 | < 0.001 |

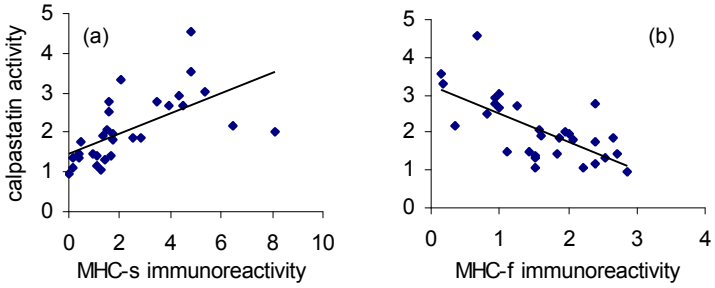


**Figure 1** Regression of (a) MHC-s immunoreactivity and (b) MHC-f immunoreactivity with calpastatin activity. Immunoreactivity is expressed as OD/unit tissue, activity as $10^9$ fluorescence units/kg.

**Conclusions** The immunochemical data classifies LD and TFL as fast muscles, ST as intermediate, and TZ and SS as slow muscles. Consistent with previous studies (Singh et al., 1997) calpastatin activity was shown to differ between the five muscles studied. Calpastatin inhibitory activity was found to be highly associated with the immunoreactivity of MHC-slow and -fast isoforms. The data suggests that the variability of calpastatin inhibitory activity between and within muscles could be partly influenced by muscle fibre type distribution.

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**Relationship between meat quality and blood acid-base measurements in pigs**

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**Introduction** Pale soft exudative pork (PSE) is a major problem affecting swine industries worldwide that results in significant economic loss because it reduces processing and saleable product yields. The PSE condition results from a rapid rate of muscle glycolysis early postmortem and a rapid drop in muscle pH while the temperature of the carcass is still high. Stress prior to slaughter can increase the rate of glycolysis and postmortem acidification. Blood acid-base has been used as an indicator of stress in pigs. The objective of this experiment was to investigate the relationship between blood acid-base status at slaughter and fresh meat quality in pigs.

**Materials and methods** A total of 40 commercial hybrid pigs were used. Pigs were transported from the farm a distance of two km to the University of Illinois Meat Science Laboratory on the morning of slaughter and held in lairage for 30 min. without food but with access to water. At the time of exsanguination, 1.5 ml of blood was collected in a 3 ml sodium lithium heparinized tube. The blood was assayed for pH, $PCO_2$, $PO_2$, $SO_2$, $TCO_2$, $HCO_3$, base excess, and lactate using an i-STAT Clinical Analyzer (i-STAT Corp., Princeton, NJ). Quality measurements taken on the longissimus at the 10$^{th}$ rib included pH at 45 min and 24 h postmortem, subjective scores for color (6 point scale; 1=pale pinkish gray to white, 6=dark, purplish red), marbling (continuous scale; 1=1% intramuscular lipid, 10=10% intramuscular lipid; NPPC, 2000), and subjective firmness (5 point scale; 1=soft, 5=very firm; NPPC, 1991), Minolta color (L*, a*, and b* values), 24-h drip loss, cooking loss, and Warner-Bratzler shear force.

**Results** There was a substantial range in individual animal values for both blood parameters and meat quality measurements, including blood lactate (mean = 10.7 Φmol/L ∀ 3.22, range 3.1 to 17.7), blood pH (mean = 7.25 ∀ 0.073, range 7.06 to 7.44), longissimus pH at 45 min. (mean = 6.15 ∀ 0.280, range 5.40 to 6.62), and 24 h (mean = 5.48 ∀ 0.075, range 5.19 to 5.60), Minolta L* (mean = 53.0 ∀ 4.65, range 45.6 to 62.9), drip loss (mean = 5.49 % ∀ 2.830, range 1.45 to 12.84), and shear force (mean = 2.66 kg ∀ 0.560, range 1.90 to 4.20). As expected, the correlations between the blood acid-base measurements were generally strong (Table 1). A number of meat quality measurements were also strongly related. However, correlations between measures of blood-acid base and pork quality were generally weak and non-significant ($P$>0.05). Blood lactate was positively correlated to longissimus cooking loss ($P$<0.05) and there was a trend ($P$<0.10) for a relationship between blood $TCO_2$ and ultimate pH and between blood $PO_2$ and drip loss.

**Table 1.** Correlations among blood gas parameters and meat quality measurements [a]

| Variable | Ult. pH | Color | Marbling | Firmness | L* | Drip loss | Cook loss | Blood Lactate | Blood $TCO_2$ | Blood pH | Blood $PCO_2$ | Blood $P0_2$ | Blood $HCO_3$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ult. pH | 0.25 | | | | | | | | | | | | |
| Marbling | 0.35[c] | 0.68[e] | | | | | | | | | | | |
| Firmness | 0.36[c] | 0.82[e] | 0.68[e] | | | | | | | | | | |
| L* | -0.40[d] | -0.73[e] | -0.64[e] | -0.78[e] | | | | | | | | | |
| Drip loss | -0.36[c] | -0.54[e] | -0.59[e] | -0.73[e] | 0.69[e] | | | | | | | | |
| Cook loss | -0.30[b] | -0.37[d] | -0.33[c] | -0.46[e] | 0.39[d] | 0.63[e] | | | | | | | |
| Blood | | | | | | | | | | | | | |
| Lactate | -0.17 | -0.11 | -0.11 | -0.12 | 0.06 | 0.19 | 0.31[c] | | | | | | |
| $TCO_2$ | 0.26[b] | 0.15 | -0.06 | -0.02 | -0.00 | 0.12 | -0.13 | -0.27[b] | | | | | |
| Blood pH | 0.06 | -0.10 | 0.06 | 0.02 | 0.03 | -0.16 | -0.23 | -0.79[e] | -0.09 | | | | |
| $PCO_2$ | 0.05 | 0.18 | 0.01 | -0.2 | -0.03 | 0.18 | 0.11 | 0.49[e] | 0.55[e] | -0.87[e] | | | |
| $P0_2$ | -0.12 | -0.14 | -0.04 | -0.15 | 0.10 | 0.26[b] | 0.33 | 0.24 | -0.35[c] | -0.05 | -0.13 | | |
| $HCO_3$ | 0.25 | 0.10 | 0.01 | -0.07 | 0.04 | 0.13 | -0.14 | -0.37[c] | 0.98[e] | 0.04 | 0.43 | -0.33[c] | |
| Base-excess | 0.25 | 0.09 | 0.07 | -0.01 | 0.03 | 0.04 | -0.22 | -0.63[e] | 0.86[e] | 0.41[d] | 0.07 | -0.32[c] | 0.92[e] |

[a] Significant correlation: [b] $P$<0.10; [c] $P$<0.05; [d] $P$<0.01; [e] $P$<0.001.

**Conclusions** Relationships between blood acid-base status at slaughter and pork quality were generally very weak. Further research is needed to evaluate these relationships in pigs handled with differing pre-slaughter management practices and under commercial conditions, where increased variation in both pork quality and blood acid-base status could be anticipated.

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# Comparison of immunochemical and histochemical analysis of fibre type distribution in ovine skeletal muscles

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**Introduction** The characterisation of muscle fibres has become increasingly important as the proportion of slow and fast fibre types are known to influence the biochemical and physiological properties of muscle during postmortem tenderisation (Ouali and Talmant, 1990). Current histochemical methods are labour intensive, time consuming and hazardous, requiring rapid freezing of samples in isopentane cooled in liquid nitrogen. The purpose of this study was to investigate an alternative immunochemical approach for identifying fibre types by examining the expression of slow myosin heavy chain (MHC-s) and fast myosin heavy chain (MHC-f) and comparing the data with classical histochemical techniques. Five different ovine skeletal muscles with known differences in fibre types distribution were studied.

**Materials and methods** Six 12 month old Mule x Charolais rams were slaughtered by electrical stunning and severance of the carotid arteries. *Longissimus dorsi* (LD), *tensor fasciae latae* (TFL), *semitendinosus* (ST), *trapezius* (TZ) and *supraspinatus* (SS) muscle samples were removed from the left side of each carcass within 5 min postmortem and immediately frozen in either isopentane (pre-cooled to -150°C with liquid nitrogen) for histochemical analysis and in liquid nitrogen for immunochemical analysis. Serial tissue sections (20 µm) were pre-incubated in an acidic buffer (pH 4.6) before staining for myosin ATPase activity (Brooke and Kaiser, 1970). Fibre types in 10 randomly selected areas were counted under light microscopy and the percentage of each fibre type in the different muscles was calculated (Figure 1). Proteins from muscle homogenates were electrophoresed and transferred to nitrocellulose before being immunoprobed with monoclonal anti-MHC-s and MHC-f (1:1000) antibodies (Novacastra). Blots were reprobed with rabbit anti-actin antibody (1:500, Sigma) to correct for protein load. Bands were visualised by the CDP-star detection system (Amersham; Figure 2) and quantified by Quantity One image analysis software (BioRad). Differences in fibre type distribution between muscles were analysed by one-way analysis of variance. Regression analysis was used to correlate MHC-s and MHC-f expression with the histochemical data.

**Results** Fibre type characterisation by both methods revealed significant differences in MHC-f ($p < 0.05$) and MHC-s expression ($p < 0.001$) and the percentage of type I slow and type II fast fibres ($p < 0.001$) between the muscles (Table 1). *Trapezius* and SS were shown to be composed predominantly of slow fibres as evidenced by the MHC-s expression and percentage of slow type I, whilst MHC-f and fast type II fibres were found to be more abundant in TFL and LD. Regression analysis indicated a significant positive association between immunochemical expression of MHC-s and MHC-f and the percentage of slow type I ($r^2 = 0.52$, $p < 0.001$) and fast type II ($r^2 = 0.24$, $p < 0.01$) fibres, respectively.

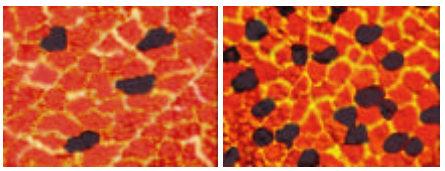


**Figure 1** Myosin ATPase (pH 4.6) stained tissue sections (20 µm) of (a) LD and (b) SS muscle. Slow type I fibres stained dark and fast type II fibres stained light.

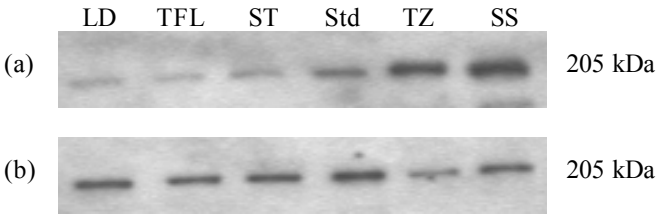


**Figure 2** Representative western blots of whole muscle samples taken from a single animal. The samples were probed with (a) MHC-s (1:1000) and (b) MHC-f (1:1000). Std =standard

**Table 1** Effects of muscle on fibre type composition

| | LD | TFL | ST | TZ | SS | sed | p |
|---|---|---|---|---|---|---|---|
| MHC-s (OD) | 1.3 | 0.4 | 1.5 | 4.6 | 4.1 | 0.7 | < 0.001 |
| MHC-f (OD) | 2.0 | 2.0 | 1.8 | 1.3 | 0.8 | 0.4 | < 0.05 |
| % type I | 8.9 | 8.1 | 16.8 | 54.5 | 44.1 | 6.7 | < 0.001 |
| % type II | 91.1 | 91.9 | 83.2 | 45.5 | 55.9 | 6.7 | < 0.001 |

**Conclusions** The results of the present study demonstrate that different ovine muscles vary in their composition of slow and fast fibre types. The positive correlation between the results obtained by both methods show that immunochemical MHC-slow and fast expression has the potential to be used to estimate the fibre type composition of different ovine skeletal muscles.

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# Effect of feeding diets with excess dietary leucine to finishing pigs on growth and carcass characteristics, meat quality, and intramuscular fat levels

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**Introduction** Concerns over low levels of intramuscular fat and poor eating quality of meat from modern lean lines of pigs have focused attention on approaches to increasing the marbling fat content of pork. A number of techniques to increase intramuscular fat by manipulation of the nutrient composition of the diet have been evaluated. These have included feeding of protein deficient diets and of excess dietary leucine levels (Cisneros et al., 1996). Leucine is a ketogenic amino acid, the carbon skeleton of which can be used to synthesize fatty acids in muscle. In a previous study (Cisneros et al., 1996) feeding excess dietary leucine increased intramuscular fat and also improved muscle color. This study was carried out to validate those findings.

**Materials and methods** The study was carried out as a completely randomized design using a 2 x 2 factorial arrangement. The treatments were: l) dietary leucine level (normal [1.22%] vs high [3.22%]) and 2) gender (barrows vs gilts). Forty crossbred pigs (Duroc × Yorkshire) were reared from 78.4 ± 3.46 to 114.8 ± 7.30 kg BW over a 39-day study period. The study was carried out in a fully-slatted facility with pigs being housed and fed individually and provided 1.6$m^2$/pig of floor space. Pigs were given ad libitum access to diets that were formulated using corn and soybean meal to exceed the nutrient requirements of NRC (1998). The normal leucine diet contained 13.2% CP, 0.79% lysine, 1.22 % leucine and 3,420 kcal ME/kg and the high leucine diet contained 15.2% CP, 0.79% lysine, 3.22 % leucine and 3,400 kcal ME/kg. The increased leucine was achieved using L-leucine. Pigs were slaughtered at a commercial facility and carcass and meat quality measurements were taken 24 hrs postmortem. Carcass measurements included carcass weight, and 10$^{th}$ rib fat depth and longissimus area. Quality measurements taken on the longissimus included subjective color, firmness and marbling scores (scale: 1 = pale, soft and devoid of marbling to 5 = dark, firm and abundant or greater marbling), and Hunter color (L*, a*and, b*). Drip loss was determined on a loin chop and the fat and water content of the longissimus was analyzed using standard procedures. Data were analyzed using PROC GLM procedures of SAS (SAS Inst. Inc., Cary, NC) with the model including effects of leucine level, gender, and their interaction.

**Results** There were no treatment interactions (P>0.05). Pigs fed high leucine diets were lighter (P<0.05) at the end of study and grew more slowly (P<0.05) than the pigs fed the diet with the normal leucine level. However, the high leucine level increased marbling score (P<0.05) and muscle fat content (P<0.05) compared to the normal leucine level. There was no effect of dietary leucine level on other meat quality measurements. As anticipated barrows grew faster, were fatter, and had higher marbling and intramuscular fat levels and firmer muscle scores than gilts (Table 1).

Table 1. Effect of dietary leucine level and gender on growth performance and carcass and meat quality.

| Items | Leucine level | | | Gender | | |
|---|---|---|---|---|---|---|
| | Normal | High | SEM | Barrow | Gilts | SEM |
| Initial BW, kg | 78.9 | 79.1 | 0.79 | 79.7 | 78.2 | 0.79 |
| Final BW, kg | 115.4 [a] | 111.1 [b] | 1.39 | 115.9 [a] | 110.6 [b] | 1.39 |
| ADG, g | 930 [a] | 829 [b] | 32.2 | 928 [a] | 830 [b] | 32.2 |
| ADFI, kg | 2.89 | 2.75 | 0.072 | 3.05 [a] | 2.58 [b] | 0.072 |
| Gain: Feed | 0.32 | 0.30 | 0.009 | 0.30 | 0.32 | 0.009 |
| Hot carcass wt, kg | 83.9 | 81.1 | 1.55 | 82.6 | 82.4 | 1.55 |
| Killing out percent | 73.1 | 73.0 | 0.08 | 72.9 | 73.0 | 0.08 |
| Backfat thickness, 10$^{th}$ rib, mm | 19.2 | 18.3 | 1.16 | 20.9 [a] | 16.7 [b] | 1.13 |
| Longissimus area, 10$^{th}$ rib, $cm^2$ | 60.3 | 57.0 | 1.50 | 54.7 [a] | 62.6 [b] | 1.49 |
| Subjective color score | 3.2 | 3.5 | 0.17 | 3.4 | 3.3 | 0.17 |
| Subjective firmness score | 3.3 | 3.3 | 0.14 | 3.5 [a] | 3.1 [b] | 0.14 |
| Subjective marbling score | 3.2 [b] | 3.9 [a] | 0.24 | 4.0 [a] | 3.1 [b] | 0.24 |
| Ultimate pH | 5.7 | 5.7 | 0.06 | 5.7 | 5.7 | 0.05 |
| Drip loss, % | 2.6 | 2.5 | 0.50 | 2.0 | 3.1 | 0.50 |
| Longissimus moisture, % | 73.7 | 73.4 | 0.24 | 73.1 [a] | 74.0 [b] | 0.24 |
| Longissimus fat, % | 2.4 [b] | 3.4 [a] | 0.33 | 3.7 [a] | 2.2 [b] | 0.33 |

[ab] Within a row and comparison, means without a common superscript letter differ (P < 0.05).

**Conclusions** The results of the present study suggests that feeding excess leucine increased intramuscular fat content but reduced growth rates and had no effect on muscle color.

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# Rumen biohydrogenation of polyunsaturated fatty acid sources and their effect on plasma fatty acid status in sheep

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**Introduction:** Increasing the *n-3* polyunsaturated fatty acid (PUFA) content of ruminant products may be important in reducing the incidence of cardiovascular diseases in man. Previous experiments suggest that a-linolenic acid (C18:3*n*-3) in the form of whole linseed is extensively biohydrogenated both *in vitro* (Cooper *et al*., 2001) and *in vivo* (Wachira *et al*., 2000) and that some form of protection is required. By contrast the long chain PUFA's in fish oils appear less susceptible to biohydrogenation (Wachira *et al*., 2000). The objective of the present study was to quantify the extent to which *n-3* PUFA from different sources were biohydrogenated in the rumen and to determine the degree to which they were incorporated into plasma lipids.

**Materials and Methods:** Six ruminally and duodenally canulated wether lambs aged eight months, were used in a 6×6 Latin square design. Six diets based on straw were formulated to provide similar fat levels (60g/kg DM) from different sources; Diet 1: Linseed oil (C18:3*n*-3; a-linolenic acid), Diet 2: Linseed oil absorbed onto vermiculite (C18:3); Diet 3: Formaldehyde/formic acid treated whole linseed (C18:3); Diet 4: Fish oil (C20:5 *n*-3; eicosapentaenoic, EPA and C22:6 *n*-3 docasahexenoic acid, DHA); Diet 5: Omega 3 (C20:5 and C22:6) and Diet 6: a 50:50 mix of marine algae and fish oil (C20:5 and C22:6). Fatty acid sources used in diets 2, 3 and 5 were supplied by Trouw, UK. Following a 21 day adaptation period, data were collected over a 7 day measurement period. Dry matter flow at the duodenum was estimated using Yb-acetate and Cr-EDTA as the solid and liquid phase markers respectively. Feed, duodenal digesta and plasma samples were analysed for fatty acid (FA) composition. The experiment was analysed by ANOVA.

**Results:** Intake of a-linolenic acid was highest in diets 1, 2 and 3, whilst intake of EPA and DHA was highest in diets 4, 5 and 6. Duodenal FA flow was 89, 106, 115, 90, 109 and 94% of intake for diets 1 to 6, respectively. The biohydrogenation of linoleic acid was 83-91% in all diets whilst the biohydrogenation of a-linolenic acid was lowest in animals offered diet 2 at 74% followed by diet 5 at 83%, whilst that of the other diets was approximately 88%. The biohydrogenation of EPA and DHA ranged between 62-87% and 62-80% respectively in diets 4, 5 or 6. No significant difference in plasma C18:3 was observed in animals fed diets 2 or 3 compared with 1. By contrast, plasma EPA and DHA was significantly higher ($p<0.001$) in sheep offered the algae/fish oil diet than the fish oil diet.

**Table 1.** *Fatty acid intake, biohydrogenation and plasma fatty acids*

| | Linseed oil | Linseed/ Vermiculite | Formic Linseed | Fish oil | Omega 3 | Algae/fish | s.e.d |
|---|---|---|---|---|---|---|---|
| **Intake (g/d)** | | | | | | | |
| C18:2-linoleic | 15.6 | 14.9 | 15.4 | 10.4 | 10.5 | 9.1 | |
| C18:3-linolenic | 32.1 | 30.6 | 24.0 | 4.2 | 6.8 | 4.2 | |
| C20:5-EPA | - | - | - | 3.1 | 2.1 | 2.1 | |
| C22:6-DHA | - | - | - | 5.1 | 4.6 | 14.1 | |
| Total FA | 69.8 | 58.0 | 57.4 | 64.5 | 69.2 | 71.6 | |
| Total duodenal FA flow (g/d) | 62.4 | 61.7 | 66.3 | 58.2 | 75.7 | 67.2 | 6.86 |
| **Biohydrogenation (%)** | | | | | | | |
| C18:2 | 90.7 | 83.8 | 83.8 | 90.9 | 87.2 | 90.0 | 1.66 |
| C18:3 | 92.0 | 74.4 | 85.3 | 87.3 | 83.1 | 88.0 | 4.47 |
| C20:5 | - | - | - | 86.7 | 62.5 | 76.9 | 3.79 |
| C22:6 | - | - | - | 79.1 | 62.5 | 61.1 | 4.58 |
| **Plasma FA (%)** | | | | | | | |
| C18:2 | 14.8 | 17.2 | 16.9 | 9.4 | 12.0 | 8.9 | 0.67 |
| C18:3 | 6.7 | 7.0 | 5.6 | 1.4 | 2.2 | 0.9 | 0.55 |
| C20:5 | 2.8 | 3.2 | 3.5 | 8.2 | 7.7 | 11.8 | 0.70 |
| C22:6 | 2.7 | 3.4 | 3.0 | 3.8 | 5.5 | 8.2 | 0.61 |
| **Total FA (mg/ml)** | 1.05 | 0.87 | 0.98 | 0.98 | 0.83 | 0.89 | 0.053 |

**Conclusion:** Linoleic and a-linolenic acid were extensively biohydrogenated in the rumen. Feeding linseed oil with vermiculite significantly reduced biohydrogenation but did not alter plasma C18:3. The long chain PUFA's were more resistant to biohydrogenation in the rumen and the algae/fish oil diet significantly increased plasma EPA and DHA concentration compared to the fish oil diet and could be used to increase *n*-3 PUFA's in sheepmeat.

**Acknowledgements:** We are grateful to DEFRA, ABN Ltd,, Roche products, Tesco Stores Ltd, and Pedigree Petfoods for funding this project.

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# Fatty acid composition and quality of muscle from steers fed ruminally protected lipid

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**Introduction** Recommendations to improve the UK diet suggest an increase in the ratio of polyunsaturated to saturated fatty acids (P:S ratio) and a higher consumption of n-3 polyunsaturated fatty acids (PUFA). Ruminant meats have a poor P:S ratio, approximately 0.1 compared to the recommendation of 0.4-1.0 for the whole diet. However, the ratio of C18:2 n-6/C18:3 n-3 (the n-6:n-3) is well within the recommended value of <4.0 at approximately 2 and ruminant muscle also supplies longer-chain n-3 PUFA. By feeding lipid in a formaldehyde cross-linked protein matrix, rumen biohydrogenation can be avoided and the tissue PUFA level increased but with potential effects on oxidative shelf-life, colour and flavour of the meat. This trial investigated the fatty acid composition and quality of meat produced by feeding a protected lipid supplement (PLS).

**Materials and Methods** Samples of *m.longissimus* were obtained from 24 Charolais cross steers (initial liveweight 528 (se 6.3) kg, 8 per treatment) that had been fed for 90 days a forage: concentrate (60:40, DM basis) diet with one of three supplements containing equal amounts of lipid derived from 1 Megalac (Mega); 2. Megalac + PLS (1:1 lipid basis) and 3. PLS. The PLS was a spray dried emulsion of soya bean, linseed and sunflower oil treated with formaldehyde. The PLS lipid was 4% of diet DM. The ratio of linoleic acid: α-linolenic acid was 11:1 in Megalac and 2:1 in PLS. Samples for lipid analysis were taken 48h post-mortem and frozen. Muscle, conditioned for 10 days at 1ºC in vacuum packs, was taken for organoleptic assessment and used to determine shelf-life during simulated retail display by measuring colour and lipid oxidation (TBARS). Lipids were extracted with chloroform: methanol, separated into neutral and polar fractions on silicic acid columns and the fatty acid composition determined by gas-liquid chromatography. Fatty acid results were analysed by ANOVA with diet as the main factor.

**Table 1.** Proportions (x100) of C18:2 n-6 and C18:3 n-3 in muscle neutral and polar lipids

| Diet: | Mega | Meg+PLS | PLS | sed | P |
|---|---|---|---|---|---|
| Polar lipids | | | | | |
| C18:2 n-6 | 15.0 | 27.5 | 31.7 | 1.34 | <0.001 |
| C18:3 n-3 | 2.3 | 3.3 | 3.1 | 0.17 | <0.001 |
| Neutral lipids | | | | | |
| C18:2 n-6 | 1.2 | 3.2 | 4.7 | 0.37 | <0.001 |
| C18:3 n-3 | 0.5 | 1.3 | 1.9 | 0.13 | <0.001 |

**Results** Feeding the PLS increased the proportions of C18:2 n-6 and C18:3 n-3 in muscle neutral lipids and polar lipids (Table 1) with little change in the longer chain PUFA in phospholipids. These changes decreased the colour shelf-life (Fig 1) shown by the decrease in depth of red colour (saturation) and increased lipid oxidation (TBARS) during display (Fig 2). However, effects on flavour attributes were small: values for "abnormal" flavour (score 1 none? 100very strong) were 11.4$^{ab}$, 7.9a and 15.4b, sed 2.12, P<0.01 for Mega, Mega + PLS and PLS respectively and for fatty/greasy were 17.1$^{a}$, 22.3$^{b}$ and 20.1$^{ab}$, sed 1.77, P<0.05. Scores for overall liking and beef flavour were not affected.

**Fig 1.** Effect of diet on colour shelf-life (± sem) of beef loin steaks displayed in MAP

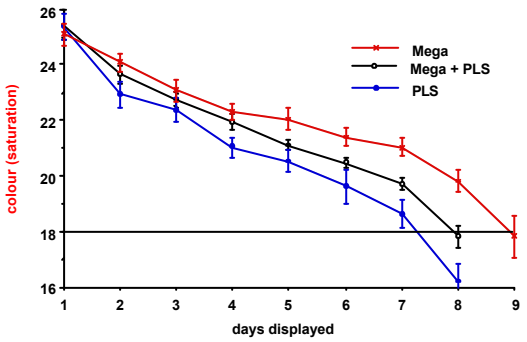
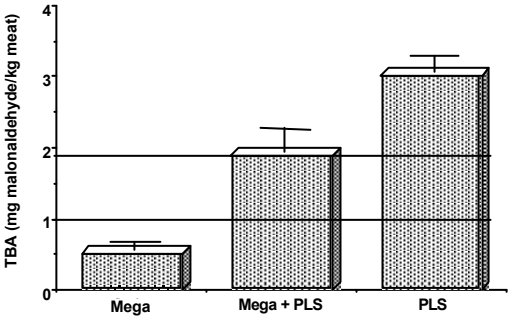



**Fig 2.** Effect of diet on mean TBARS (±sem) of beef loin steaks after 10d display in MAP




**Conclusions** This study demonstrates that increasing the content of linoleic and α-linolenic acid in beef can be achieved with minimal effects on eating quality (flavour) although the small changes in shelf-life (colour and lipid oxidation) suggest that further increases in PUFA concentrations would be unacceptable.

**Acknowledgements** We are grateful to DEFRA, ABN Limited, Pedigree Petfoods, Rare Breed Survival Trust, Roche Products and Tesco Stores Limited for supporting this work and Rumentek Industries, Parkside Australia for providing the PLS.

# Effect of feeding diets rich in n-3 PUFA at different stages of the production period on the PUFA composition of intramuscular fat in Belgian Blue double-muscled bulls

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**Introduction** Belgian beef production is mainly using double-muscled Belgian Blue animals finished indoors on all-concentrate diets or high-concentrate/maize silage diets. In relation to consumer health and in order to improve the "functional food" value of beef, it is attempted to increase its n-3 PUFA content using linseed or grass and grass silage in the fattening diet. The aim of this study was to investigate the effect of feeding grass or linseed at different stages of the production period on the intramuscular PUFA content of Belgian double-muscled beef.

**Materials and methods** Twenty three Belgian Blue bulls (mean (SD): 336 (50) kg liveweight) were divided into three groups (straw-concentrate (SC), grass silage-concentrate (GC) and maize silage-concentrate (MC)), fed different rations during periods of last growth, prefattening and fattening until slaughtering (mean (SD): 681 (30) kg liveweight) (Table 1). Concentrates were cereal and beet pulp based with added linseed (n-3 source) or without, formulated at the same level. During the first two periods, roughage was fed *ad libitum* and concentrates at low amounts (ca. 2 kg/d at the last growth period and 1 % of live weight daily in the prefattening period). Muscle samples of M. *longissimus thoracis*, M. *semitendinosus* and M. *triceps brachii* were taken 24 h *post mortem.* Samples were immediately vacuum packed and frozen at –20 °C until fatty acid analysis, as described by Raes *et al.* (2001). Data were analysed by a 2-way ANOVA with the factors diet and muscle (SPSS version 9.0).

**Results and Discussion** PUFA composition (mg/100 g muscle) is shown in Table 2 as mean values for the three muscles. The total intramuscular fat content for the three groups was very low (0.7 g fat/100 g meat) and was slightly higher for the GC group. No differences in total PUFA content was observed between the groups. However, the GC group had a significantly higher content of n-3 fatty acids (C18:3, C20:5, C22:5) and a significantly lower content of n-6 fatty acids (C18:2, C20:4, C22:4). These animals received a n-3 rich diet throughout the whole trial. No difference was observed in the total n-3 content between the SC and MC group, although these were submitted to a shift from dietary n-3 to n-6 and n-6 to n-3 fatty acids respectively. However, the SC group has a significantly higher content of long chain n-3 fatty acids (C20:5 and C22:5) compared to the MC group. This suggests that the supply of n-3 fatty acids during the pasture period was important for the formation and incorporation of the long chain n-3 fatty acids in the cell membranes. Cell membranes contain phospholipids, rich in PUFA and with a slow turnover. The supply of linolenic acid in the fattening period of the MC group after a period with mainly n-6 fatty acids in the diet did not result in the same levels of longer chain n-3 PUFA compared with the SC group that received mainly n-6 fatty acids in the fattening diet. Including linseed in the fattening diet (GC and MC group) increased the linolenic acid content and decreased the n-6/n-3 ratio. This ratio was lower for the GC group compared to the MC group, again demonstrating the additional effect of receiving n-3 fatty acids in the preceding growing phases before the fattening phase.

**Table 1** Experimental set-up and composition of the diets

| | SC | GC | MC |
|---|---|---|---|
| Animals (n = ) | 7 | 8 | 8 |
| Last growth phase | Pasture | Pasture | Indoors |
| (± 340-440 kg)[a] t=[b] | 70 | 70 | 70 |
| Concentrate | + linseed | + linseed | - linseed |
| Roughage | n-3 / grass | n-3 / grass | n-6 / maize silage |
| Prefattening phase | Indoors | Indoors | Indoors |
| (± 440-520 kg) t= | 92 | 98 | 56 |
| Concentrate | + linseed | + linseed | - linseed |
| Roughage | n-6 / GPS[c] | n-3 / grass silage | n-6 / maize silage |
| Fattening phase | Indoors | Indoors | Indoors |
| (± 520-680 kg) t= | 139 | 134 | 83 |
| Concentrate | - linseed | + linseed | + linseed |
| Roughage | Straw | n-3 / grass silage | n-6 / maize silage |
| C/R[d] | 100/0 | 70/30 | 80/20 |

[a] Mean live weight at start and end of the period; [b] Duration (days) [c]GPS = triticale silage [d]C/R = Concentrate/Roughage ratio on DM basis mixed at feeding and fed at maximal intake level

**Table 2** Intramuscular fatty acid composition (mg/100 g muscle)

| | SC | GC | MC | RSD |
|---|---|---|---|---|
| Sum | 620[a] | 738[b] | 632[a] | 167 |
| PUFA | 213 | 198 | 204 | 29.4 |
| Σn-6 | 179[a] | 133[b] | 165[a] | 27.0 |
| C18:2 n-6 | 133[a] | 101[b] | 123[a] | 24.4 |
| C20:4 n-6 | 32.2[a] | 24.6[b] | 29.6[c] | 3.81 |
| C22:4 n-6 | 2.49[a] | 1.61[b] | 2.77[a] | 0.92 |
| Σn-3 | 35.1[a] | 63.6[b] | 38.3[a] | 6.74 |
| C18:3 n-3 | 12.2[a] | 33.8[b] | 19.0[c] | 4.41 |
| C20:5 n-3 | 8.13[a] | 11.7[b] | 6.79[c] | 1.59 |
| C22:5 n-3 | 13.3[a] | 16.1[b] | 11.3[c] | 1.91 |
| C22:6 n-3 | 1.18[a] | 1.28[b] | 0.90[ab] | 1.10 |
| n-6/n-3 | 5.04[a] | 2.11[b] | 4.37[c] | 0.67 |
| P/S | 0.72[a] | 0.50[b] | 0.67[a] | 0.23 |

[a,b,c] diffrent superscripts in the same row indicate significant differences (P<0.05)

**Conclusions** The results suggest that the intramuscular fatty acid composition is not only affected by the fatty acid composition of the fattening ration, but also by the ration offered during the preceding growing phases of the animals, especially with respect to the longer chain fatty acids of the n-3 series. Including linseed in the diet results in a higher C18:3 n-3 content and a lower n-6/n-3 PUFA ratio.

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# Effect of production systems on carcass quality characteristics of lambs

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**Introduction** Our objective was to provide basic information that can be used to direct sheep production to produce meat, which meets consumer demands. Here, we present the results of a series of studies that were designed to assess the effect of different production systems on carcass quality characteristics of lambs from Greek dairy breeds of sheep.

**Material and methods** A total of 480 lambs of the Karagouniko breed, an indigenous Greek dairy breed of sheep, were used. They had been randomly allocated into four groups (n=120 lambs each). After weaning the lambs were reared under different conditions to produce four different types of carcasses (Type I, II, III and IV). The first group of lambs (entire males) were slaughtered at weaning (50 days of age) to produce Type I carcasses. For Type II carcasses, lambs (entire males) were weaned at 48 days and subsequently group fed indoors with 500 g/day/head of a concentrate ration ((192g Crude Protein (CP)/kg DM and 11.3 MJ Metabolizable Energy (ME)/kg DM)) and 500g/day/head of Lucerne hay (182g CP/kg DM and 6,4 MJ ME/kg DM). They were slaughtered at 128 days of age. The third group of lambs (castrated) were weaned at 54 days of age and finished on irrigated sown pasture (*Lolium perenne* + *Trifolium repens*). They were slaughtered after 114 days on pasture to produce Type III carcasses. Type IV carcasses were produced from castrated lambs weaned at 46 days and subsequently fed indoors for 38 days with a concentrate ration (192g CP/kg DM and 11,3 MJ ME/kg DM) and wheat straw, both offered *ad libitum.* All lambs were weighed weekly. Carcass conformation, fatness (1 to 5 point scale) and colour were classified by visual assessment according to the European Association of Animal Production standardised methods. Subsequently, the carcasses were split down the spinal cord and the left hand side (LHS) was cut in quarters. Ribeye measurements (values A and B), muscle colour evaluation (CIELAB values, L*, a* and b*) and pH measurements were made in the cut surface of *the longissimus lumborum* muscle. All data were analysed by one-way ANOVA. Means were compared with Tukey's test.

**Results** Table 1 shows the measurements of carcass characteristics of lambs reared under different systems. There were significant differences (P<0.001) in carcass yield between the different types of carcasses. There were also significant (P<0.001) differences between carcass types in conformation and fatness with Type I being the worst. However, the majority of all carcasses types were classified as regular and ordinary. The ribeye measurements were significantly (P<0.001) different between the four carcasses types with Type II having the higher values. Muscle colour measurements (L* values) indicated that the brightest meat was that of milk-fed lambs (Type I) whereas darker colours were observed in Type III and IV carcasses (P<0.001). Type III and IV carcasses had also significantly (P<0.001) higher a* and lower b* values, suggesting a more red colour when compared to the other two carcasses types. Values of pH did not indicate large variations between different carcass types; only pH values of Type I were significantly (P<0.001) different from others.

**Table 1** Carcass characteristics by group (n=120) of lambs (means ± SD)

| | | Type I | Type II | Type III | Type IV |
|---|---|---|---|---|---|
| LWT (Kg)[1] | | 15.9±1.32$^a$ | 33.4±1.46$^b$ | 31.8±1.78$^c$ | 24.1±1.27$^d$ |
| Carcass yield (%)[2] | | 52.9±2.40$^a$ | 46.3±1.68$^b$ | 42.8±2.10$^c$ | 45.9±1.86$^b$ |
| Conformation | | 1.0±0.18$^a$ | 2.3±0.52$^b$ | 2.2±0.74$^b$ | 2.1±0.45$^b$ |
| Fatness | | 3.2±0.52$^a$ | 2.4±0.51$^b$ | 2.3±0.59$^b$ | 2.7±0.45$^c$ |
| Ribeye (mm) | A | 47.9±3.26$^a$ | 57.7±3.14$^b$ | 51.7±3.67$^c$ | 50.3±2.88$^c$ |
| | B | 23.7±2.23$^a$ | 25.1±2.42$^b$ | 22.4±3.48$^c$ | 21.2±2.37$^c$ |
| Colour (muscle) | L* | 37.7±2.51$^a$ | 34.4±2.73$^b$ | 32.0±2.26$^c$ | 32.1±2.41$^c$ |
| | a* | 6.9±1.3$^a$ | 7.7±0.96$^b$ | 8.0±0.88$^b$ | 7.3±0.96$^c$ |
| | b* | 6.6±0.74$^a$ | 6.4±0.90$^a$ | 5.7±0.78$^b$ | 4.5±0.67$^c$ |
| pH | | 5.7±0.16$^a$ | 5.5±0.10$^b$ | 5.5±0.09$^b$ | 5.5±0.09$^b$ |

[1]*Live weight at slaughter,* [2]*Calculated on a cold carcass basis.*

**Conclusions**
Results of this study provide options available to the Greek sheep industry to produce specific types of lamb carcasses dependent upon the particularities of the production systems practiced in Greece and described here.

**Acknowledgements:** The work was supported by the E.C. (DG VI) project No FAIR CT 96-1768.

# $D^9$-desaturase activity in the mammary gland of lactating dairy cows

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**Introduction** The $\Delta^9$-desaturase system (steroyl-CoA desaturase) involves the addition of a *cis* double bond between carbons 9 and 10 of fatty acids. The conversion of stearic acid ($C_{18:0}$) to oleic acid (*cis*-9 $C_{18:1}$) is the predominant precursor:product of this enzyme system; conversion of saturated fatty acids (SFA) to mono-unsaturated fatty acids (MUFA) is important in determining the fluidity of milk. In previous studies (Lock & Garnsworthy 2001), we have shown that more than 50% of the oleic acid occurring in milk is produced in the mammary gland via $\Delta^9$-desaturase. We also found that the conversion of *trans*-11 $C_{18:1}$ to *cis*-9, *trans*-11 conjugated linoleic acid (CLA) accounted for ~80% of milk fat CLA. Increasing the activity of $\Delta^9$-desaturase offers the opportunity of increasing the MUFA content of milk, especially oleic acid, while decreasing its SFA content, as well as increasing the CLA content of milk. Lock & Garnsworthy, (2001) also reported that there were significant differences between individual cows with regard to milk fat CLA content. In an earlier study (Lock & Garnsworthy, 2000) we found that the CLA content of milk varied throughout the year, with highest values occurring when cows received fresh pasture. In view of the significant contribution of $\Delta^9$-desaturase to the CLA and MUFA content of milk, the objective of the work reported here was to investigate individual animal and dietary variation in $\Delta^9$-desaturase activity in the mammary gland of lactating dairy cows.

**Materials and methods** Two experiments were carried out. In the first (Lock & Garnsworthy, 2000), a total of 433 daily milk samples were taken from the University of Nottingham's commercial dairy herd between December 1997 and June 2000. All animals followed the same dietary regime throughout the study. During the winter months, a total mixed ration of grass and maize silages, brewers grains, cereals, soya and dairy concentrates was fed. Through the summer months, fresh grass was fed, with increasing levels of buffer feeding given as the summer progressed. In the second experiment (Lock & Garnsworthy, 2001) eight cows received 4 diets that differed only in their supply of linoleic and linolenic fatty acids to the rumen, in a 4x4 Latin square design. For both experiments milk fatty acid profiles were obtained and reported in Lock & Garnsworthy (2000, 2001). The ratio of $C_{14:1}$ to $C_{14:0}$ was used to calculate $\Delta^9$-desaturase activity since all of the $C_{14:0}$ in milk fat is produced via *de novo* synthesis in the mammary gland, so all $C_{14:1}$ is produced via $\Delta^9$-desaturase. In the first experiment data were subjected to analysis of variance, with sampling month and individual animal used as the factor. In the second experiment, data were subjected to analysis of variance, using diet and individual animal as factors.

**Results** In the first experiment the $C_{14:1}$:$C_{14:0}$ ratio averaged 0.062 throughout the year. The months of May and June had significantly higher ($P<0.05$) ratios than all other months, indicating substantial differences in $\Delta^9$-desaturase activity. Throughout the summer months of May, June and July the $C_{14:1}/C_{14:0}$ ratio averaged 0.072; the other months averaged 0.058 (Table 1). Individual cow significantly affected ($P<0.001$) the $C_{14:1}$:$C_{14:0}$ ratio, varying between 0.039 and 0.121. In the second experiment diet did not significantly affect the $C_{14:1}$:$C_{14:0}$ ratio, which averaged 0.085 across all four treatments, indicating that there was no substantial effect of dietary linoleic and linolenic acid on $\Delta^9$-desaturase activity in the mammary gland. Analysis of variance showed that individual cows had significantly different ($P<0.001$) $\Delta^9$-desaturase activities (Figure 1). $C_{14:1}$:$C_{14:0}$ ratios ranged between 0.067 and 0.103. Across dietary treatments, cows tended to remain either in the higher or lower areas of $\Delta^9$-desaturase activity.

**Table 1** Changes in the $C_{14:1}$:$C_{14:0}$ ratio throughout the year

| Feb | Mar | Apr | May | Jun | Jul | Aug |
|---|---|---|---|---|---|---|
| 0.058[a] | 0.059[a] | 0.057[a] | 0.079[b] | 0.073[b] | 0.064[a] | 0.059[a] |
| Sep | Oct | Nov | Dec | s.e.d | Sig | |
| 0.056[a] | 0.057[a] | 0.058[a] | 0.059[a] | 0.0044 | *** | |

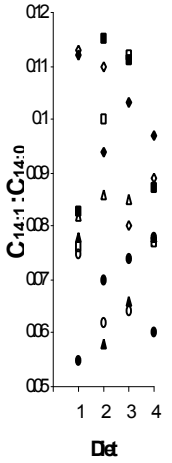


**Figure 1** Individual cow variation in $\Delta^9$-desaturase

**Conclusions** Results show that there is significant variation between individual animals in the activity of $\Delta^9$-desaturase in the mammary gland of lactating dairy cows. Seasonal changes in the $C_{14:1}$:$C_{14:0}$ ratio would suggest that there is a dietary effect on $\Delta^9$-desaturase activity, since activity was greatest in the summer months when cows were mainly receiving fresh pasture. This was not found in the second experiment, because the diets were iso-energetic and nitrogenous and differed only in their supply of individual fatty acids. Because the majority of CLA occurring in milk is produced via $\Delta^9$-desaturase, it would appear that previously reported seasonal and animal variations in the CLA content of milk may be largely explained by changes in $\Delta^9$-desaturase activity in the mammary gland. What influences this activity is not well understood, but findings reported here indicate that activity may be influenced by nutrition and genetics.

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# Conjugated linoleic acid and trans C18:1 in muscle and adipose tissue of lambs fed supplements containing n-3 polyunsaturated fatty acids

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**Introduction** Conjugated linoleic acids (CLA) occur in the milk and tissues of ruminants. Although cis-9, trans-11 CLA is an intermediate in the biohydrogenation of linoleic acid in the rumen, duodenal flows of CLA are very low (Scollan *et al*, 2001) and most CLA appears to be synthesised in tissues from trans-11 C18:1 (vaccenic acid) (Griinari *et al*, 2000). Trans C18:1 isomers are also produced in the rumen and their amounts are increased when the levels of dietary unsaturated fatty acids are raised. We have investigated the relationship of tissue levels of CLA and trans C18:1 in three breeds of lambs fed supplements of linseed or fish oil to increase trans C18:1.

**Materials and Methods** 72 eight week old male lambs of three breeds: Suffolk x Lleyn, Soay and Friesland x Lleyn, initial liveweight of 26, 12 and 24 kg respectively, were individually penned and randomly allocated to one of four iso-energetic, iso-nitrogenous pelleted diets based on dried grass containing different added fats (total fat level 6 g/kg DM). The dietary fats were: 1. Megalac; 2. Linseed; 3. Fish oil; 4. A Linseed/fish oil mixture (1:1, oil basis). Animals were fed *ad libitum* and slaughtered at approximately half the breed mature live weight (44, 21 and 44 kg respectively). Samples of m. *longissimus dorsi* and subcutaneous back fat were taken for fatty acid analysis by gas-liquid chromatography. Trans C18:1 isomers were not completely resolved and are reported as a single value. Results were analysed by ANOVA with breed, fat and breed x fat interaction as the main factors. There were no interactions. Linear regression was used to investigate the relationship of CLA to total precursor assessed as CLA + C18:1 trans.

**Results** The proportion of trans C18:1 was higher in adipose tissue than muscle total lipids and there was no significant effect of breed (Table 1). However, proportions of CLA were lowest in both tissues from Suffolk lambs and highest in Soays with intermediate concentrations in the Freislands. The Lin/fish diet resulted in the highest proportion of trans C18:1 in both muscle and adipose tissue whereas Linseed and Fish oil produced similar concentrations; approximately double that in animals fed Megalac. The effects of dietary fat on CLA differed markedly from those on trans C18:1: Megalac and Fish oil resulted in similar proportions of CLA whereas linseed and lin/fish gave higher but similar levels. Although the deposition of CLA was low when Fish oil was fed, linear regressions of muscle CLA against trans C18:1 + CLA across feeds within breed were highly significant for the Soay and Friesland lambs ($y = 0.1543x + 0.1737$, $R^2$ 0.6891; $y = 0.1444x + 0.2991$, $R^2$ 0.7915 respectively). However the regression for the Suffolks had a lower slope indicating a decreased conversion of trans C18:1 to CLA ($y = 0.078x + 0.5682$, $R^2$ 0.2986) and a poorer correlation. Despite the relationships for muscle, in adipose tissue it is clear that Fish oil alone, while increasing trans C18:1, resulted in a poor deposition of CLA.

**Table 1** *Effect of breed and dietary fat on the proportion (x100) of trans C18:1 and CLA in lamb muscle and adipose tissue*

| Fatty acid | | Suffolk | Soay | Friesland | sed | P |
|---|---|---|---|---|---|---|
| C18:1 trans | Muscle | 6.8 | 6.5 | 6.2 | 0.46 | NS |
| CLA | | 1.2 | 1.5 | 1.3 | 0.10 | <0.05 |
| C18:1 trans | Adipose tissue | 10.8 | 10.1 | 10.1 | 0.74 | NS |
| CLA | | 1.1 | 1.9 | 1.5 | 0.12 | <0.001 |

| | | Megalac | Linseed | Fish oil | Lin/fish | sed | P |
|---|---|---|---|---|---|---|---|
| C18:1 trans | Muscle | 3.8 | 6.6 | 7.0 | 8.6 | 0.53 | <0.001 |
| CLA | | 1.0 | 1.6 | 1.1 | 1.7 | 0.12 | <0.001 |
| C18:1 trans | Adipose tissue | 5.4 | 10.8 | 10.6 | 14.1 | 0.86 | <0.001 |
| CLA | | 1.1 | 1.9 | 1.1 | 1.9 | 0.13 | <0.001 |

**Conclusions** The deposition of CLA in muscle and adipose tissue of lambs is affected by both breed and dietary lipid but the cause remains unclear. The poor conversion of increased trans C18:1 with fish oil feeding might result from inhibition of $\Delta^9$-desaturase by EPA or DHA or possibly a different relationship between the levels of CLA and trans C18:1 in the rumen.

**Acknowledgements** We would like to acknowledge funding from DEFRA and support from Roche Products Ltd, International Fishmeal and Oil Manufacturers Association and Trident Feeds.

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# Manipulating lamb conjugated linoleic acid content and stearoyl coenzyme A desaturase mRNA by either a grass or concentrate feeding regime

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**Introduction** Conjugated Linoleic Acid (CLA) is a mixture of isomers of linoleic acid implicated with numerous health promoting properties. These include anti-carcinogenicity (*cis*-9, *trans*-11 isomer), anti-atherogenicity and increasing the lean:fat ratio (*trans*-10, *cis*-12 isomer). CLA is produced naturally by all ruminant animals through the incomplete biohydrogenation of linoleic acid within the rumen. Alternatively, it can be made endogenously by stearoyl coenzyme A desaturase (SCD) from vaccenic acid (VA) (Griinari *et al,* 2000). It has been well documented that cows fed on a grass diet produce more *cis*-9, *trans*-11 in milk than those fed on a concentrate based regime (Kelly *et al,* 1998) but to date, little work would appear to have been undertaken to determine if this is the case with sheep tissues. In the present study, a grass based diet was compared with a concentrate diet in order to determine whether the CLA content of adipose tissue differed and if so, which isomer and what mechanisms may be involved.

**Materials and Methods** Twenty four Mule x Charolais ewe lambs approximately 8 weeks old (average initial liveweight 28.6 kg) were randomly allocated to one of 3 treatment groups: (diet 1) grass nuts, (diet 2) restricted barley-oats based concentrate diet to give the same growth rate as the grass nuts or (diet 3) the same concentrate diet fed approaching *ad-libitum.* The trial was conducted over a 7-week period. At slaughter, samples of adipose tissue (peri-renal, omental and subcutaneous) and abomasal contents were taken and snap frozen in liquid nitrogen. Lipid was extracted using a 2:1 chloroform:methanol solution. Prior to GC analysis using a 60m BPX70 column, adipose tissue was methylated by base methylation and abomasal contents by combined base-acid methylation. SCD mRNA levels were determined using a ribonuclease protection assay. Two way ANOVA was used to compare differences between the diets and depots.

**Results** For all parameters mesaured there was no significant interaction between diet and depot. Lambs fed diet 3 had significantly higher SCD mRNA concentrations than those fed diet 1 in all three adipose tissue depots ($p<0.001$), see Daniel *et al* (2002). Similar proportions of *cis*-9, *trans*-11 and VA were found in the abomasal contents of all three treatment groups but more *trans*-10, *cis*-12 was found in those fed diets 2 and 3 ($p<0.01$). However, lipid content of the abomasal digesta was significantly higher for diet 1 (44.2, 24.6, 22.8 mg/g for diets 1, 2, 3 respectively, s.e.d. 6.05, $p=0.003$). There was no significant difference (NS) in the proportion of VA found in the adipose tissue depots on any treatment (Table 1). *Cis*-9, *trans*-11 was significantly higher in all adipose tissue depots of animals fed grass nuts (Table 1). Conversely, concentrate-fed lambs produced significantly more *trans*-10, *cis*-12 than grass nut fed lambs in all adipose tissue depots (Table 1).

**Table 1** Effect of diet and depot on CLA content

| | Fatty acid x $10^{-3}$ (moles/mole FAME) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *cis*-9, *trans*-11 | | | *trans*-10, *cis*-12 | | | VA | | |
| diet | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| subcut | 13.06 | 10.21 | 6.91 | 0.00 | 0.31 | 0.00 | 53.90 | 58.80 | 61.50 |
| omental | 10.45 | 8.3 | 5.97 | 0.00 | 0.35 | 0.11 | 69.90 | 70.90 | 65.40 |
| perirenal | 8.01 | 4.85 | 3.26 | 0.05 | 0.48 | 0.14 | 71.10 | 71.60 | 62.70 |
| | diet $p<0.001$ | | | diet $p<0.001$ | | | diet NS | | |
| | depot $p<0.001$ | | | depot NS | | | depot NS | | |
| s.e.d. | 0.549 | | | 0.064 | | | 4.210 | | |

Feeding grass nuts produced the greatest proportion of *cis*-9, *trans*-11 in adipose tissue. This data suggests that the *cis*-9, *trans*-11 content of the tissue is not related to the expression of the SCD gene in adipose tissue. The increase in tissue *cis*-9, *trans*-11 is more likely to be attributable to differences in ruminally produced *cis*-9, *trans*-11 or VA as indicated by the abomasal lipid content.

**Conclusion** These results confirm that feeding grass produces greater amounts of tissue *cis*-9, *trans*-11 as compared to a concentrate diet. However, the mechanism by which this is achieved would not appear to be directly related to SCD gene expression.

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# Modelling the odour of cooked meat *in vitro* using different fatty acids

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**Introduction** The odour/flavour of beef from animals raised on cereal concentrates differs from that of forage fed animals and this is thought to be caused, at least in part, by differences in fatty acid composition (cereals, high in linoleic acid, C18:2; grass, high in linolenic acid, C18:3). During cooking, the thermal oxidation of fatty acids produces aroma volatiles and intermediates (Mottram, 1991) that modify the Maillard reaction between amino acids and reducing sugars. In this study, we have investigated the reactions that naturally occur in the muscle during cooking by heating together a sugar (ribose), a sulphur amino acid (cysteine) and several unsaturated fatty acids to evaluate the organoleptic contribution of fatty acids to meat aroma development.

**Material and methods** Six different mixtures were created from combinations of fatty acids, oleic (C18:1), linoleic (C18:2) and a-linolenic (C18:3) with or without a mixture of L-cysteine and D(-)ribose. For each model system, 0.5 mmol of each compound was placed in 100ml capacity screw capped *Duran* borosilicate glass bottles with 20ml of pyrophosphate buffer 0.2M at pH 5.5. The reaction mixtures were heated at 140$^{o}$C under pressure for 30 minutes and allowed to cool. Reaction mixtures were diluted 1/100 and 10ml were transferred into 50ml capacity narrow mouth *Volac* amber bottles with glass stoppers for odour assessment. They were presented to a 10-member trained taste panel. Panellists assessed the odour at room temperature, in individual booths with red light to mask colour differences, for the descriptors in Table 1 using an unstructured scale where 0 meant absence of the odour and 100 meant a very intense odour.

**Results** Adding cysteine (c) and ribose (r) to the heated fatty acids (Table 1) markedly increased the scores for the meat-associated descriptors, 'meaty' and 'fatty', indicating the importance of the Maillard reaction in the development of meat odour. However, panellists did not find differences between 18:2+c+r and 18:3+c+r in 'meaty' or 'fatty' although the higher unsaturation of C18:3 makes it more reactive. The term 'fatty' was clearly distinguished from the odour of 'cooking oil', that was associated with heating C18:2 alone and that was decreased when cysteine and ribose were present. Similarly, the descriptor 'oily' was associated with heated C18:1 and was also decreased in the presence of cysteine and ribose although Melton (1990) had associated 'oily' with meat with high content of C18:2. The 'fish oil' odour of heated a-linolenic acid was also significantly decreased by cysteine and ribose but the overall scores for this odour were much lower than a 'linseed oil/putty' like oxidised odour of C18:3. Although cysteine and ribose decreased this odour, it remained high. A high value for the descriptor 'sweet' was expected with C18:2 due to an increase in 6-?-dodecenolactone (Melton, 1990) but this was not different between the mixtures.

**Conclusion** It is possible to imitate 'meaty' odours by cooking different mixtures of fatty acids *in vitro* together with a sugar and a sulphur amino acid. 18:2 and 18:3 produced similar meaty aromas in the presence of cysteine and ribose but alone they produced different odours, 18:2 scoring most for 'cooking oil' and 18:3 for 'fishy' and 'linseed'. These notes were reduced when cysteine and ribose were included.

**Table 1.** *Odour profile assessed by the test panel in reaction mixtures of fatty acids (C18:1, C18:2, C18:3), amino acid (cysteine, c) and sugar (ribose, r).*

| | 18:1 | 18:2 | 18:3 | 18:1+c+r | 18:2+c+r | 18:3+c+r | sed | |
|---|---|---|---|---|---|---|---|---|
| Meaty | 0.5 $^{a}$ | 2.9 $^{a}$ | 0.4 $^{a}$ | 21.4 $^{c}$ | 15.7 $^{bc}$ | 11.0 $^{b}$ | 3.52 | *** |
| Fatty | 0.5 $^{a}$ | 2.1 $^{ab}$ | 0.3 $^{a}$ | 10.4 $^{c}$ | 6.2 $^{abc}$ | 8.4 $^{bc}$ | 3.22 | ** |
| Cooking oil | 1.8 $^{a}$ | 30.1 $^{b}$ | 2.8 $^{a}$ | 5.6 $^{a}$ | 7.0 $^{a}$ | 5.4 $^{a}$ | 4.96 | *** |
| Oily | 18.8 $^{b}$ | 6.7 $^{a}$ | 8.7 $^{a}$ | 3.6 $^{a}$ | 2.9 $^{a}$ | 1.4 $^{a}$ | 3.80 | ** |
| Cod liver/fish oil | 0.1 $^{a}$ | 0.4 $^{a}$ | 6.4 $^{b}$ | 0.1 $^{a}$ | 0.1 $^{a}$ | 2.7 $^{a}$ | 1.50 | *** |
| Linseed/putty | 1.0 $^{a}$ | 2.3 $^{ab}$ | 30.4 $^{c}$ | 1.6 $^{a}$ | 2.8 $^{ab}$ | 12.4 $^{b}$ | 5.10 | *** |
| Sweet | 4.6 | 3.4 | 2.9 | 4.3 | 2.2 | 0.7 | 1.46 | n.s. |

$^{a,b,c}$ *Means in the same row are significantly different (** p<0.01; ** p<0.001).*

**Acknowledgements** This research is supported by a Marie Curie Fellowship of the European Community programme Quality of Life under contract number QLK1-CT-1999-51147. The authors wish to thank Anne Baker and Sue Hughes for their technical assistance.

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# Effect of diet on vitamin E metabolism and meat quality in lambs

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**Introduction** Supplementation of animal diets with supranutritional levels of vitamin E is nowadays considered advisable because it extends shelf life by delaying myoglobin and lipid oxidation. However, vitamin E absorption and deposition in ruminants fed on concentrate based diets is reported to be variable. This work examines the effect of concentrate or grass silage based diets on vitamin E concentrations in plasma, muscle and liver and meat quality in lambs.

**Materials and methods** Four groups of eight Suffolk × Charollais wether lambs, previously grazed, were individually penned and allocated by live weight to a 2 × 2 factorial experimental design. Two levels of vitamin E (60 and 500 mg/kg DM α-tocopheryl acetate) both in excess of the usual 20 mg/kg DM in commercial diets and two diets (concentrates and grass silage supplemented with concentrates (mixed diet) were used. Diets were fed *ad libitum* for approximately two months following a five day adaptation period. To obtain a slaughter weight of 40kg, the concentrate-fed lambs entered the experiment at a live weight of 25±1.6 kg (SED) and mixed diet fed lambs at a live weight of 32±3.3kg (SED). Blood samples were obtained weekly for plasma vitamin E and monthly for glutathione peroxidase (GSHPx) and creatine kinase (CK) determination. After slaughter, muscle (m. *semimembranosus*) and liver samples were taken for measuring vitamin E using HPLC. For colour assessment during display and oxidative stability 15mm thick leg steaks were conditioned for 6 days in vacuum at 0°C, repacked in modified atmosphere ($O_2$:$CO_2$, 0.75:0.25) and displayed under light (cool white fluorescent illumination with 700lx, 16hr on/8hr off) at 4°C for 6 days. M. *semimembranosus* colour was determined daily using CIELAB L*a*b* colour space and oxidative stability was determined as thiobarbituric acid reacting substances (TBARS) after 3 and 6 days of display**.**

**Results** At the end of the trial plasma vitamin E levels of concentrate-fed lambs on the low vitamin E treatment reached values lower than 0.3 μg/ml that is considered the threshold between adequacy and deficiency (Figure 1). Growth rate and GSHPx and CK values did not indicate a deficiency state and GSHPx and CK values improved during the feeding period in all lambs. Vitamin E levels were higher at the end of the trial than at the start for lambs on all the other treatments. Lambs on the mixed diet had higher muscle and liver vitamin E levels despite the shorter feeding period. There was a moderate correlation between plasma and muscle and between plasma and liver vitamin E levels ($R^2$ = 0.52 and 0.64 respectively) but a good correlation between muscle and liver vitamin E levels ($R^2$=0.77). Colour was little affected by dietary vitamin E level but the lambs fed the mixed diet had a more intense muscle red colour during the entire display period. Lipid oxidation was very high for the concentrate fed lambs on the 60mg treatment and negligible for all other treatments. Lipid oxidation values reflected the muscle vitamin E concentrations (Table 1).

**Figure 1.** *Plasma vitamin E levels during the feeding period. Concentrates (C) and mixed diet (M).*

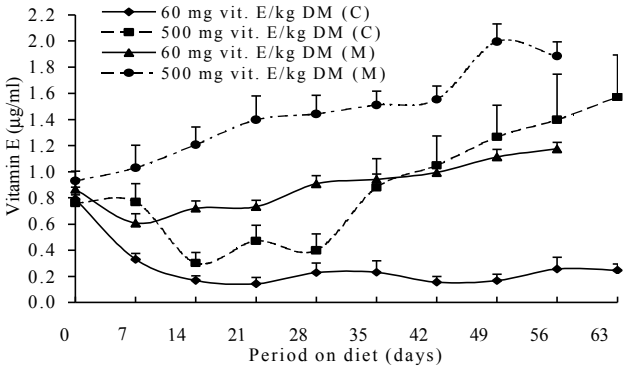


**Table 1.** *Diet and vitamin E effect on plasma, muscle and liver vitamin E concentrations at slaughter, colour and lipid oxidation (mean values)*

| | Vitamin E (mg/kg DM) | | | | |
|---|---|---|---|---|---|
| | 60 (C) | 500 (C) | 60 (M) | 500 (M) | sem |
| Plasma vit. E (μg/ml) | 0.22[a] | 1.57[b] | 0.98[b] | 1.93[b] | 0.175 |
| Muscle vit. E (μg/g) | 1.01[a] | 3.41[b] | 2.88[b] | 4.67[b] | 0.251 |
| Liver vit. E (μg/g) | 1.36[a] | 10.00[b] | 6.89[b] | 16.54[c] | 0.948 |
| Redness (a*) Day 0 | 19.63 | 19.70 | 19.86 | 20.07 | 0.516 |
| Redness (a*) Day 3 | 18.11 | 18.43 | 18.79 | 18.49 | 0.414 |
| Redness (a*) Day 6 | 16.20 | 16.76 | 17.26 | 17.06 | 0.404 |
| TBARS Day 3[1] | 0.94[b] | 0.05[a] | 0.17[a] | 0.06[a] | 0.076 |
| TBARS Day 6[1] | 1.98[b] | 0.07[a] | 0.24[a] | 0.09[a] | 0.132 |

[1] mg of maloaldehyde/kg muscle [a, b, c] Numbers with different superscript letters differ significantly, p<0.05

**Conclusions** Muscle and plasma vitamin E concentrations are only moderately correlated indicating that plasma vitamin E levels are not always a reliable indicator of the actual muscle vitamin E levels. Concentrate based diets require supranutritional vitamin E supplementation to obtain adequate plasma, muscle and liver vitamin E levels. Vitamin E supplementation is more effective when grass silage is included in the diet and high quality meat can be produced even though muscle vitamin E levels do not exceed the limit of 3.3 μg/g considered necessary for optimum meat quality (Faustman *et al*., 1989).

**Acknowledgements** We acknowledge the financial support of DEFRA, Tesco Stores Ltd, Roche Products Ltd, ABN Ltd and Pedigree Petfoods. E. Kasapidou is grateful to the EU for the provision of a Marie Curie PhD fellowship.

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# Relationships between skatole and androstenone in Large White and Meishan pigs

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**Introduction** Boar taint is an offensive odour and flavour in the meat from some (perhaps 0.05) entire male pigs. It occurs when high concentrations of skatole and androstenone are reached in fat tissue. Boar taint is more common in some breeds than others and as part of an investigation into its genetic basis (Doran *et al*., 2001), we have studied skatole, androstenone and testosterone relationships in Large White and Meishan cross bred pigs.

**Materials and Methods** Approximately 90 entire male pigs were used, either Large White x Landrace (LW) or Meishan x Landrace (M). They were fed a standard concentrate feed containing 14MJDE and 10g lysine/kg for the time taken for the LW to reach 100kg live weight. Both breeds were therefore the same age at slaughter. Blood plasma and liver samples were obtained at slaughter and backfat samples one day latter. Plasma testosterone and androstenone, backfat skatole and androstenone and liver skatole concentrations were measured by HRGC using the procedures described in Doran *et al* (2000) and Wiseman *et al* (1999).

**Results** LW were heavier than M of the same age (77.5 vs 63.7 kg carcass weight ± 0.97 SEM) yet had thinner backfat (11.5 vs 14.6mm P2 fat thickness ± 0.85 SEM). Skatole and androstenone concentrations in backfat were much greater in M as was liver skatole although this difference was smaller than in backfat. Plasma testosterone and androstenone were significantly higher in M. Figure 1 shows a curvilinear relationship between androstenone and skatole with values on the steeply ascending part of the curve being predominantly LW. A plot of log skatole against androstenone shows that the 2 breeds can be considered as a continuum, with low values for both compounds in LW and high values in M.

Table 1. Backfat and liver skatole and androstenone (mg/kg ± SEM) and plasma androstenone and testosterone (nmoles/l± SEM)

| | Backfat | | Liver | Plasma | |
|---|---|---|---|---|---|
| | skatole | androstenone | skatole | androstenone | testosterone |
| LW | 0.125 (0.024) | 0.808 (0.033) | 0.048 (0.004) | 25.0 (5.57) | 20.4 (2.06) |
| M | 0.616 (0.092) | 1.225 (0.050) | 0.072 (0.015) | 36.7 (7.60) | 26.5 (2.42) |
| Signif. | *** | *** | *** | * | *** |

**Conclusions** In our earlier paper we showed that Meishan cross pigs had higher skatole concentrations in backfat than Large Whites (Doran *et al*, 2001). These results show Meishans also have higher concentrations of both testosterone and androstenone and raise the possibility of an interaction between these compounds in the overall control of boar taint.

Figure 1. Skatole and androstenone concentrations in backfat

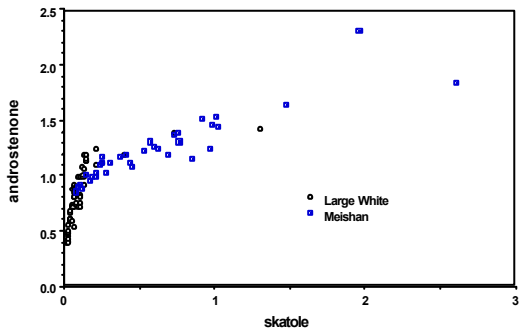
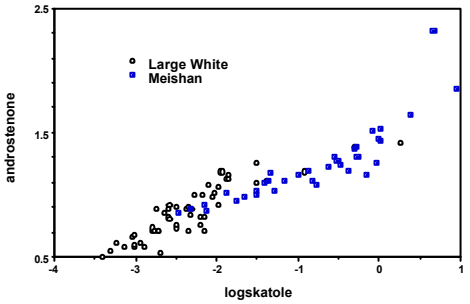



Figure 2. Log skatole and androstenone concentrations in backfat.

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**Acknowledgements** This work was funded by MLC and the Danish National Committee for Pigs.

# The estimation of chemical, physical and sensory parameters of homogenized fresh pork eye muscle by near infrared reflectance spectroscopy (NIRS)

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**Introduction** This study was based on research to identify particular pig breeds, which produce high quality eating pork. Duroc in particular is thought to improve meat quality of progeny when crossed with Large White/Landrace (LW/Lr) hybrid dams by altering the intra-muscular fat (IMF) content of the lean, which is positively related to eating quality (McGloughlin *et al*., 1988). The extraction of IMF is slow and laborious with harmful solvents involved. Eichinger and Beck (1991) have successfully used NIRS to measure IMF ranging from 1-11% in 39 beef carcases. Ground beef and pork samples have also been predicted for fat, water and protein by NIRS, with prediction errors of 0.82-1.49%, 0.94-1.33% and 0.35-0.70% respectively (Togersen *et al*.,1999). Instrumental measurements of pork are accepted as indicators of tenderness. Sensory variables such as juiciness, tenderness and flavour are important characteristics for the consumer. Therefore the objective of this study was to explore the potential of NIRS to estimate the chemical, physical and sensory parameters of homogenized fresh pork eye muscle.

**Materials and methods** Samples from the loin of 88 70kg carcasses were obtained 24 h after slaughter and frozen. The samples were from the progeny of first cross Large White/Landrace dams and eight different sire types (Duroc (Dr), Landrace (Lr), Large White (LW), Dr/Lr, Dr/LW, Lr/LW, Dr/Lr/LW and Dr/Lr/Pietrain (P)). These samples were allowed to thaw overnight in a cool room at 4°C. The eye muscle area of each chop was measured, then homogenized and brought to room temperature. The homogenized sample was packed in duplicate in a quarter cup and scanned on a NIRSystems 6500 scanning spectrophotometer. Log 1/Reflectance data were collected at 2nm intervals. The homogenized sample was then analysed for dry matter, nitrogen and IMF. Physical assessment (shear force) of the eye muscle was estimated using an Instron. Low shear scores were indicative of tender pork samples. Sensory assessment was carried out on four cooked samples per sire type. Twenty trained panellists scored the samples on a scoring system of 1-8 (1 = extremely acceptable, 8 = extremely unacceptable) for juiciness, tenderness and flavour. Average scores were calculated for each sample. NIR calibrations were formed by regressing the laboratory and physical data and sensory scores against the spectral data using the modified partial least squares regression technique and standard normal variate and detrend scatter correction. Cross-validation was used to obtain the optimum equations without over-fitting. Optimum calibrations are those which produced the lowest standard errors of cross validation (SECV).

**Results** The NIRS calibrations were good for oven dry matter and nitrogen with cross validation errors representing only 1.3 and 1.6 % error of the mean. The $R^2$ and 1-VR (coefficient of determination for validation) were particularly good for oven dry matter while the coefficients for nitrogen dropped from 0.84 to 0.75 indicating a lack of robustness in the equation. The IMF equation appears to be very robust with very little change in errors or correlation coefficients between calibration and cross validation, however a SECV of 2.86 is too large an error for prediction purposes. This large error is probably a reflection of the IMF database as the majority of samples had IMF values in the lower end of the range. Equations based on physical and sensory parameters were extremely poor.

**Table 1** Parameter range, and means plus NIRS calibration statistics for homogenized eye muscle of fresh pork

| Parameter | n | Range | Mean | SEC | $R^2$ | SECV | 1-VR |
|---|---|---|---|---|---|---|---|
| Oven dry matter (g/kg) | 79 | 234-312 | 264.1 | 2.96 | 0.96 | 3.40 | 0.94 |
| Nitrogen (g/kg) | 81 | 33.2-40.0 | 36.46 | 0.48 | 0.84 | 0.61 | 0.75 |
| IMF (g/kg dm) | 79 | 10.6-68.4 | 22.41 | 2.74 | 0.89 | 2.86 | 0.89 |
| Shear (kg/cm$^2$) | 71 | 1.70-3.72 | 2.59 | 0.455 | 0.018 | 0.469 | 0.000 |
| Tenderness Score | 31 | 2.65-5.60 | 4.01 | 0.69 | 0.03 | 0.73 | 0.00 |
| Juiciness Score | 31 | 3.30-4.25 | 4.10 | 0.17 | 0.89 | 0.37 | 0.43 |
| Flavour Score | 31 | 2.85-4.55 | 3.65 | 0.25 | 0.29 | 0.27 | 0.21 |

SEC = standard error of calibration

**Conclusion** NIRS has the potential to accurately and rapidly estimate the dry matter and total nitrogen in fresh homogenized pork. The prediction of IMF could be improved by the addition of more samples. The NIRS predictions of physical and sensory variables were not acceptable.

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# The effects of level of bedding provision and time in lairage on the contamination of hides of finished cattle with potentially zoonotic bacteria.

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**Introduction** Many cases of food-borne illness in the UK are related to the consumption of contaminated meat products. This has highlighted the importance of adopting hygienic procedures throughout the meat production chain, including the farm and abattoir environment (Pennington, 2000). Many factors are known to affect the hygienic condition of finished cattle (Davies *et al.*, 2000) and various husbandry practices may be used to improve cleanliness at slaughter. However, the extent to which abattoir practices contribute to the contamination of cattle hides is not known. Overnight lairage of cattle prior to slaughter is a common practice in the UK, but the extent to which this affects bacterial contamination of the hide remains to be determined. This study investigated the effects of providing additional straw bedding during lairage on the microbiological cleanliness of cattle during overnight lairage.

**Methods** A total of 64 Limousin-sired cattle, finished on a silage-based ration, were used in the study. Cattle were transported to an abattoir in two groups of 32, an on arrival, penned in eight pens of four animals. For each group, four of the lairage pens were provided with adequate levels of straw bedding (Adequate), this being the usual amount used in the abattoir. The remaining four pens were bedded with double the amount of straw used in the adequately bedded pens (2 x Adequate). Swab samples were taken from the brisket of cattle at four time points – immediately after unloading (Time 0; 1400 hrs), and after four (Time 4; 1800 hrs), eight (Time 8; 2200 hrs) and 17 hours (Time 17; 0700 hrs) in lairage. At each of these time points two animals per pen were sampled, with the left and right-hand side of each animal being swabbed on two separate time points, resulting in 64 samples per time point. As an index of bacterial load, Total Viable Counts (TVC) and Total *Enterobacteriacae* were enumerated using automated plate counting equipment, following plating of diluted inocula onto nutrient agar plates and incubation for 72 hours at 30°C. Data were transformed to $\log_{10}$ values, analysed using ANOVA and are expressed as a mean and standard error. Back transformed values are given in brackets.

**Results** No significant differences were observed in microbiological contamination between adequate and 2 x Adequate bedding level treatments, at Time 0, 4 or 8. However, after 17 hours in lairage, cattle kept on 2 x Adequate bedding had significantly lower bacterial counts compared with those on Adequate bedding.

***Effects of time in lairage and bedding level on microbiological contamination of cattle***

| | | **Time 0** | **Time 4** | **Time 8** | **Time 17** |
|---|---|---|---|---|---|
| $\log_{10}$ Total Viable Count | Adequate Bedding | 7.44 ±0.13 (27.5 x $10^6$) | 7.11 ± 0.21 (12.8 x $10^6$) | 6.72 ± 0.17 (5.2 x $10^6$) | 7.12 ± 0.18 (13.2 x $10^6$) |
| | 2 x Adequate | 7.07 ± 0.18 (11.7 x $10^6$) | 6.92 ± 0.17 (8.3 x $10^6$) | 6.63 ± 0.18 (4.3 x $10^6$) | 6.20 ± 0.27 (1.6 x $10^6$) |
| Effect of bedding level | | NS | NS | NS | $P < 0.01$ |
| | | | | | |
| $\log_{10}$ *Enterobacteriacae* | Adequate Bedding | 2.32 ± 0.28 (209) | 2.18 ± 0.32 (151) | 1.96 ± 0.24 (91) | 3.12 ± 0.34 (1318) |
| | 2 x Adequate | 2.09 ± 0.29 (123) | 2.48 ± 0.29 (302) | 2.33 ± 0.24 (214) | 2.09 ± 0.26 (123) |
| Effect of bedding level | | NS | NS | NS | $P < 0.05$ |

**Conclusions** The provision of straw bedding for cattle during lairage is common practice for improving animal welfare, but this study has shown that when straw is provided at double the normal rate, it also has the potential to improve the microbiological cleanliness of cattle hides prior to slaughter. Although TCS were lower in the 2 x Adequate straw treatment for the duration of the study, this only reached statistical significance after 17 hours in lairage.

**Acknowledgements** The funding of this work by the Food Standards Agency is gratefully acknowledged.

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# Genetic evaluation of Iranian Holstein dairy cows with the use of a Covariance Function Model

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**Introduction** The use of test day models has increasingly become of interest in genetic evaluation of dairy cattle. Traditionally, in most dairy cattle breeding programmes genetic evaluation of dairy sires and cows has been primarily based on using 305-day lactation yield. Since in this system of evaluation many incomplete lactation records have to be extended to complete lactations this could result in biased prediction of breeding values due to any over or under-estimation of total lactation yields. In genetic evaluation of dairy cattle, test day models have many advantages over the traditional method (Lactation Animal Model) because of the fact that they can account for the lactation curve of individual cows and take more accurate account of the effects of environmental factors influencing test day milk yield over the course of lactation. The main aim of this study was to use a Covariance Function Test day Animal Model (CFTAM) in genetic evaluation of first parity Iranian Holsteins.

**Material and Methods** A total of 53,673 monthly test day milk records collected from 6,101 Iranian Holstein dairy heifers calved between 1983 and 1995 and distributed in 174 herds from different climatic regions of Iran was used in this study. Monthly test day milk records were initially adjusted for the heterogeneous variances within contemporary groups using the procedure outlined by Ibanez *et al*., (1996). Data were subsequently subjected to a Covariance Function Test day Animal Model to estimate genetic parameters and to obtain **BLUP** breeding values of animals. In the model, fixed effect of contemporary groups of Herd-Year-Season Of Production ( $HYSOP_{it}$ **),** covariate of age of cow at test day ( $A_{ijkt}$ ), random effects of additive genetic ( $a_{jRt}$ ), permanent environment ( $pe_{jRt}$ ) and measurement error ( $ME_{ijkt}$ ) were fitted. Orthogonal Legendre Polynomial function (Kirkpatrick, 1994) was utilised in the model to take account of the variation of milk yield at two genetic and permanent environment levels during the course of lactation. The model was as follows:

$$y_{ijkt} = HYSOP_{it} + \sum_{m=1}^{2} b_m * (A_{ijkt})^m + \sum_{R=0}^{NC} g_R * f_R(t) + \sum_{R=0}^{NC} a_{jRt} * f_R(t) + \sum_{R=0}^{NC} pe_{jRt} * f_R(t) + ME_{ijkt}$$

**Results** Multivariate **REML** estimates of heritability as well as the average of genetic, phenotypic and permanent environment correlations between monthly test day milk yields are presented in Table 1. Generally, heritability of monthly test day milk yield increased from the first stages of lactation towards the middle of lactation then decreased as the lactation stage advanced. Over the period of lactation, genetic correlation ($r_A$) between monthly test day milk yields was the highest followed by permanent environment ($r_{pe}$) and phenotypic ($r_P$) correlations. For a 305-day lactation period, the average Best Linear Unbiased Prediction (**BLUP**) of breeding values of cows in CFTAM was 69.11 (Kg) and significantly greater than the corresponding average (36.31 Kg) obtained in 305-day Lactation Animal Model.

**Table 1** Genetic parameters as well as Spearman Rank correlation ($r_{T,305}$) between **BLUP** breeding values resulted from genetic evaluation based on the Covariance Function Test day and 305-day Animal Models

| | *MTD1* | *MTD2* | *MTD3* | *MTD4* | *MTD5* | *MTD6* | *MTD7* | *MTD8* | *MTD9* | *MTD10* |
|---|---|---|---|---|---|---|---|---|---|---|
| $h^2$ | 0.08 | 0.14 | 0.18 | 0.23 | 0.29 | 0.32 | 0.31 | 0.29 | 0.26 | 0.18 |
| $r_A$ | **0.77** | | | | | $r_P$ | **0.56** | | | |
| $r_{pe}$ | **0.68** | | | | | $r_{T,305}$ | **0.87 (Cows), 0.91 (Sires)** | | | |

**Conclusion** As compared to Covariance Function Model, Lactation Animal Model based on 305-day lactation records could result in a lower average **BLUP** breeding value of cows. Additionally, rank of cows and sires may also change when the genetic evaluation is carried out by a Covariance Function Model. This is mainly due to the fact that in genetic evaluation based on 305-day lactation records a part of the genetic variation among animals is removed due to lack of accounting for the shape of the lactation curve of individual cows. A Covariance Function Model is assumed to be more accurate than a 305-day Lactation Animal Model because it allows the shape of the lactation curve of individual cows differ at a genetic level and as result breeding value of animals in a given population can be predicted more precisely which in turn leads to increase genetic progress over a period of time.

**Acknowledgements** The authors are grateful to the Centre of Genetic Improvement of Livestock (Ministry of Agricultural Jihad) of Iran for supplying the data used in this study.

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# Genetic and environmental factors affecting some measures of yield and fertility in a registered Canadian Holstein dairy herd in Iran

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**Intoduction** milk production is the most important trait in dairy cattle breeding. Measures of additional traits are also important. Traits of lactation curve are important in their relation to production characteristics. The two major reasons for which dairy cows are disposed are fertility problems and low milk yield (Hansen et al.,1983). The objectives of this study were 1) To evaluate effects of some environmental factors on some measures of yield and fertility, and 2) To estimate genetic parameters for these traits applying REML procedures under sire model.

**Materials and Methods** Frist lactation records of performance test-day information for 1648 cows calving between Autumn 1989 and Summer 1994 in a registered Canadian Holstein dairy herd , located in Khorasan province,Iran, were available. Each cow had compeleted one lactation yield measured from milk weights taken twice-a-daily through the lactation.The average number of test-day milk (TDMY) per individual cow was 12. Approximately 28000 TDMY were accumulated over 6 years. Other measures of yield were peak yield (PY),milk persistency (MP),cumulative milk yield up to 90 (MY90), 180 (MY180), and 305 (MY305) days post partum. Measures of reproductive performance were number of days open (NODO), gestation length (GL), age at first calving (AAFC) and calving ease (CE).Calving ease was a nominal scale trait with four classes ( normal calving, difficult calving, abortion-calf dead, and abortion-calf alive). Data were analysed by General Linear Models procedure of the Statistical Analysis System (SAS Institute ,1992). Constants were fitted for the effects of the year, the season of calving and their interaction. Effects of birth type , sex of the calf , and calving condition were also considered for measures of fertility. Age and age at calving squared and number of days open effects were included in the model as covariables ,whenever appropriate. After correcting for the effects of these covariables,if needed, variance components were estimated for a subset of 1320 sire-identified records of 68 sires, applying REML procedures under sire model. All relationships except for paternal half sisters were ignored. The sampling variances for the estimates of heritabilities were calculated by method of Swiger et al.(1964).

**Results** Age at calving was a highly significant source of variation for TDMY up to 180 days in milk ($p<0.0001$). The season effect on TDMY increased almost linearly from calving to 180 days post partum($p<0.0001$). The number of days open had a significant effect on TDMY, MY90, MY180 and MY305 after 180 days post partum($p<0.0001$). Results were in agreement with other studies (e.g., Auran (1974) and Danell (1982)).Age and season of calving did not have significant effects on NODO. The NODO was significantly influenced by calving condition (normal or difficult calving or calving with an abortion).Heritability estimates of the traits under study are given in table 1.

**Table 1-Means and heritability estimates of the traits under study with Their corresponding standard errors**

| $h^2$ (SE) | Mean(SE) | Trait |
|---|---|---|
| 0.11 (0.09) | 2544.8 (9.2) | Milk yield up to 90 days p.p.(kg) |
| 0.26 (0.15) | 4746.1 (54) | Milk yield up to180 days p.p.(kg) |
| 0.32 (0.18) | 6545.7 (26.7) | Milk yield up to 305 days p.p.(kg) |
| 0.15 (0.10) | 31.5 (0.36) | Peak yield (kg) |
| 0.05 (0.04) | 2.92 (0.01) | Milk persistency |
| 0.04 (0.03) | 108.0 (1.4) | Number of days open |
| 0.30 (0.08) | 280.7 (0.13) | Gestation length (month) |
| 0.36 (0.20) | 27.3 (0.04) | Age at first calving (month) |
| 0.09 (0.05) | 3.83 (0.02) | Calving ease score |

**Conclusions** Environmental factors such as year and season of parturation and age at calving for most of the tratis under study , and sex of the calf, birth type and calving condition for measures of the fertility , were significant causes of observed variation. Yield traits had almost moderate heritability but measures of fertility had low heritability ,as expected. This results suggest that selection on most of the yeild traits under study will yield moderate genetic improvement in the next generation. Low heritability estimates for PY and NODO probably suggest that these traits are more influenced by the environment .

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# An analysis of the dual purpose cattle (*B. taurus x B. indicus*) lactation curve.

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**Introduction.** The shape of the lactation curve (description of milk production through lactation) which is relevant in order to predict past, current and future lactations. Curves type I show fast increment during early lactation, followed by a slow decline. However, continuous decreasing curves, named type II (Landete-Castillejos and Gallego, 2000) are frequent in tropical diary cattle, maybe due to low nutritional status (Contreras and Rincon, 1979). Curves type II could not be accurately described by Wood (1967) model. In addition, Wood's model do not describe the inner processes in the udder. Recently, Pollot (2000) presented a mechanistic model, with 3 components and an inherent biological meaning on its parameters. The objective of the present work was to compare the ability of Wood (1967) and Pollot (2000) models to describe dual purpose cattle lactation curves and provide a first description and analysis of the *B. taurus* x *B. indicus* typical lactation curve using Pollot's (2000) model.

**Materials and methods**. Forty three full lactations of dual purpose cattle from the University of Yucatán herd, from 1990 to 2000, were used. Herd was machine milked twice daily across lactation. Functions compared was Wood (1967): $Y_{(w)}=a w^{b} e^{-cw}$; Where: $Y_{(w)}$=Milk yield at week w; a, initial milk yield; b, slope of increasing phase and c; slope of decreasing phase; and Pollot (2000): $MY= [MS/(1+((1-P_o)/P_o)e^{-Gt})]-[ML/(1+((1-Q_o)/Q_o)e^{-Dt})]*[1-e^{St}]$; Where: MY= Milk yield, MS, Maximum secretion potential, product of the total number of secretory cells produced and differentiated during the course of lactation; $P_o$, Proportion of the maximum number of secretory cells present at the start of lactation. G: relative rate in cell differentiation. ML: Maximum secretion loss, $Q_o$, proportion of differentiate cells that have died by the start of lactation, D: relative rate of decline in cell numbers, and S: secretion rate (Pollot, 2000). Each lactation was fit individually, initial values for each parameter were changed to check consistency of results. Fittings were compared using the residual means square (RMS) and Run's test as indicators of the goodness of fit. Models were compared using paired t tests, and an interpretation of Pollot´s model parameters was provided.

**Results.** Fitting was better with the Pollot model (Table 1). The higher flexibility of this model was more noticeable with curves type II or irregular curves with a second surge. Wood's model was more rigid, subestimated early lactation, and overestimated late lactation. Interpretation of Pollot's model parameters (Table 2) was given as follows: Maximum secretion (MS) was obtained before the expected time if compared with dairy cattle, with a initial epithelial cell population (inferred from Po) reaching higher values between calving and lactation peak. Since cells that goes differentiated loose its capacity for further mitosis (Zwierchowsky, 1999) few (if any) increment in cell number can be expected, and combined with a quickly decline in relative rate of apoptosis (D) we obtained the short and continuous decreasing curve typical of dual purpose cattle. Secretion rate S, was almost equal to offtake, caused by calf suckling after machine milking. Thus, the biological model revealed 2 constraints on this herd as compared with specialized dairy: low cellular proliferation in mammogenesis prepartum, reflected in limited amount of cells at the beginning of lactation and high apoptosis rate.

**Table 1.** Evaluation of the ability of two models to describe dual purpose lactation curves (N=43)

| Model | RMS | Runs |
|---|---|---|
| Pollot | 0.184 ± 0.384 a | 16.42 ± 6.41 a |
| Wood | 10.587 ± 4.617 b | 9.12 ± 3.64 b |
| P | 0.0003 | 0.0003 |

RMS:Residual mean square, Runs:Run test

**Table 2.** Dual purpose lactation curve as described with a biological model (n=43)

| Variable | MS* | Po* | G* | ML* | Qo* | D* | S* |
|---|---|---|---|---|---|---|---|
| Mean | 21.062 | 0.8667 | 0.5228 | 16.469 | 0.2294 | 0.1518 | 0.9526 |
| SE Mean | 0.867 | 0.0311 | 0.0687 | 0.857 | 0.0316 | 0.0155 | 0.0157 |
| Minimum | 7.435 | 0.3753 | 0.0001 | 2.508 | 0.0000 | 0.0421 | 0.5710 |
| Maximum | 30.000 | 0.9999 | 0.9990 | 25.000 | 0.6994 | 0.4115 | 0.9999 |

*parameters from model: MY= [MS/(1+((1-Po)/Po)*e*(-Gt))]-[ML/(1+((1-Qo)/Qo)*e*(-Dt))]*[1-*e*(St)] (Pollot, 2000)

**Conclusions.** Pollot's model gave a better fitting than Wood's model when describing dual purpose cattle lactation curve. The biological model pointed out the need to study the factors that constrained mammogenesis and caused increased apoptosis under tropical conditions.

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# Heritability of mastitis and lameness in dairy cows using threshold models

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**Introduction** Improvements in management (including health care, preventative strategies and housing design) are one way to decrease the incidence of diseases. However, susceptibility to diseases is heritable and there is interest in selection for disease resitance. Genetic parameters of diseases, such as mastitis and lameness, are required so that these traits can be included in selection programmes. Phenotypes for mastitis and lameness are not expressed on a continuous scale and Gianola (1982) suggested that threshold models are more suitable for such binary traits. Whilst threshold models have been reported as appropriate for the analysis of binary traits they demand more time and greater computing power (Kadarmideen *et al*., 2000). The objectives of the current analyses were to estimate heritability and repeatability of mastitis and lameness where these traits were treated as binary traits, and to compare the estimates to those obtained in analyses that assumed the traits were continuous (i.e. that ignored the fact that they were "all or nothing" traits).

**Materials and Methods** Mastitis and lameness records on Holstein-Friesian and British-Holstein were obtained from Livestock Services UK Ltd. Both traits were recorded as binary traits with values of either 0 (no record of the disease within a year) or 1 (disease recorded at least once per year). A large proportion of mastitis and lameness records were eliminated because of inadequate pedigree information. Genetic parameters and variance components for both traits were estimated in univariate animal models using ASREML (Gilmour et al., 1998). The model included fixed effects of herd-year-season, bred and lactation number. Lactation length in days and calving age in months were included as covariates. Random effect of animal and permanent environmental effect of animal were also included. For each trait three analyses were conducted. These were trait treated as continuous (i.e. non-categorical), trait treated as binary with underlying logistic distribution and trait treated as binary with underlying standard normal distribution.

**Results** Heritability and repeatability estimates of mastitis and lameness are shown in Table 1. Heritability of mastitis where the trait was treated as a continuous variable, 0.03, is similar to the findings of Kadarmidden *et al*. (2000) of 0.04 in Holstein-Friesian cows. Heritability of lameness treated as a continuous trait was less than 0.02 which is similar to the results of Pryce *et al*. (1997) and Kadarmideen *et al*. (2000) who reported estimates of 0.03 and 0.02 respectively. Heritability of mastitis assuming an underlying standard normal distribution was 0.13 which agrees well with the estimates of Kadarmideen *et al*. (2000) who reported a heritability of 0.13 using a threshold model. Heritability of lameness, treated as a binary trait with underlying standard normal distribution was 0.14 which is in close agreement with the findings of Kadarmideen *et al*. (2000) who reported 0.08 in a threshold model. Whilst heritability could be estimated for lameness using the threshold models it should be noted that Log Likelihood did not converge in these analyses and that unrealistically high estimates of repeatability (>1.0) was obtained using the model that assumed an underlying logistic distribution.

**Table 1. Heritability (s.e.) of mastitis and lameness using threshold models**

| Data sets | No. records | Y= Continuous | Y=Binary (under-lying logistic distribution) | Y=Binary (underlying standard normal distribution) |
|---|---|---|---|---|
| Mastitis | 1412 | **0.03 (0.011)** | **0.19 (0.082)** | **0.13 (0.046)** |
| Lameness | 610 | **0.02 (0.012)** | **0.14 (0.012)** [1] | **0.14 (0.074)** [1] |

[1] Log likelihood did not converge

**Conclusion** This study supports earlier findings that heritabilities of mastitis and lameness traits are comparatively low. Heritability estimates were higher when mastitis and lamaeness were analysed in threshold models but these analyses were considerably more demanding in terms of the computing time required and there were convergence problems for analysis of lameness.

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# Production and carcase traits of progeny sired by Limousin bulls with high and below average beef values

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**Introduction** Pedigree beef cattle breeders who record with the Signet Beefbreeder service have their records analysed by a Best Linear Unbiased Prediction (BLUP). BLUP uses the records (weights and measurements) that have been recorded, for the individual and related animals, to determine the likely performance of an individual's progeny. The analysis calculates Estimated Breeding Values (EBVs) for several traits of economic value, which are the assessments of genetic merit of the animal. EBVs are calculated for 200 day weight, 400 day weight, fat depth, muscle depth and muscle score. An economic assessment, or index, is calculated using this data, called the Beef Value. The objective of this experiment was to compare the performance of dairy-bred beef calves sired by bulls with either a high (top 10% of breed) or below average (bottom 25%) Beef Value.

**Materials and Methods** In April 1999 Holstein-Friesian dairy cows in the Harper Adams University College dairy herd were inseminated with semen from the Limousin bulls; Ronick Hawk (Beef Value of LM 29) and Staveley Hurricane (Beef Value of LM 7). The calves were born from January to March 2000 and reared through to slaughter on a silage (fermented whole-crop wheat) beef system with 14 bulls and heifers per sire. The cattle were housed in straw-bedded pens. They were fed *ad libitum* fermented whole-crop wheat silage (DM 417g/kg, 11.6 MJ ME/kg DM, 80g CP/kg DM, 307g starch/kg DM) plus trough fed 170g/kg crude protein concentrates (bulls 4.0kg, heifers 2.0kg per head per day). The bulls were selected for slaughter at MLC fat class 3, heifers at fat class 4L. To allow for the analysis of carcase classification data, a numerical value was attributed to each class as follows: Conformation: -P=1, P+=2, -O=3. O+=4, R=5, -U=6, U+=7, E=8; Fat class; 1=1, 2=2, 3=3, 4L=4, 4H=5, 5L=6, 5H=7. Calving ease was assessed by the following scale: 1 = unassisted, 2 = slight assistance, no ropes, 3 = considerable help, ropes and some pulling, 4 = veterinary intervention, or considerable manipulation e.g. head back/breach, 5 = caesarian. The data was analysed with birth weight as a blocking factor, using analysis of variance.

**Results** The calves sired by the High Beef Value bull recorded significantly higher carcase weights. Whilst the analysis of the data indicated no significant differences in any of the other measured traits, the High Beef Value sired calves recorded superior daily liveweight gains, slaughter liveweights, and killing out percentage.

**Table 1** Animal performance - Bulls

| | LM 29 | LM 7 | s.e.d | Sig. |
|---|---|---|---|---|
| Gestation (days) | 285.7 | 285.2 | 1.369 | NS |
| Calving ease | 1.29 | 1.93 | 0.341 | NS |
| Birth weight (kg) | 48.77 | 48.64 | 0.650 | NS |
| Slaughter weight (kg) | 631.6 | 609.3 | 12.75 | NS |
| Days to slaughter | 486.7 | 481.9 | 7.28 | NS |
| DLWG (kg) | 1.198 | 1.165 | 0.0242 | NS |
| Carcase wt (kg) | 358.8 | 341.6 | 7.75 | * |
| Killing out % | 56.74 | 56.11 | 0.351 | NS |
| Conformation score | 5.357 | 5.286 | 0.1951 | NS |
| Fat score | 3.143 | 3.214 | 0.1646 | NS |

*P<0.05

**Table 2** Animal performance - Heifers

| | LM 29 | LM 7 | s.e.d | Sig. |
|---|---|---|---|---|
| Gestation (days) | 284.0 | 284.33 | 1.213 | NS |
| Calving ease | 1.11 | 1.22 | 0.261 | NS |
| Birth weight (kg) | 43.67 | 43.78 | 1.822 | NS |
| Slaughter weight (kg) | 505.2 | 481.8 | 16.81 | NS |
| Days to slaughter | 496.1 | 481.1 | 18.20 | NS |
| DLWG (kg) | 0.930 | 0.913 | 0.0079 | NS |
| Carcase wt (kg) | 269.7 | 254.8 | 9.85 | NS |
| Killing out % | 53.38 | 52.86 | 0.396 | NS |
| Conformation score | 5.00 | 4.78 | 0.222 | NS |
| Fat score | 3.67 | 4.00 | 0.167 | NS |

**Conclusions** The progeny from the High Beef Value sire recorded significantly higher carcase weights compared to the calves sired by the Below Average Beef Value bull. The theoretical economic difference between the progeny from the sires should have been £11.00 per calf. In this study the difference in gross margin was £17.10 per calf based on the costs prevailing at the time of the experiment.

**Acknowledgments** Financial support from the British Limousin Cattle Society is gratefully acknowledged.

# Optimisation of selection decisions in the UK Meatlinc breed of sheep

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**Introduction** Best Linear Unbiased Prediction (BLUP) estimates of breeding values (EBVs) have been routinely used for selection decisions in the UK Meatlinc (ML) population since the early nineteen nineties. This has enabled accurate selection and has allowed higher genetic gains for traits of economic relevance than in other terminal sheep breeds (MLC, 1999). However, concerns regarding increased rates of inbreeding (ΔF) by selecting exclusively on BLUP-EBVs have arisen in this small population. Dynamic rules to maximise genetic merit while ΔF is constrained to a pre-defined level using BLUP EBVs are currently available (e.g. Grundy et al 1998). They found higher gains than standard BLUP selection at the same ΔF by using these rules. The objective of this study was to investigate the potential of these procedures for optimising selection decisions under constrained inbreeding in the UK ML sheep population.

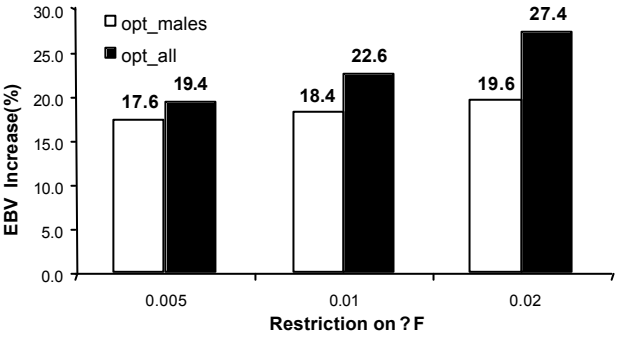
**Materials and Methods** Pedigree data and index scores for the aggregate genotype ('Lean Index', derived form BLUP EBVs) were provided by the Meat and Livestock Comission, UK (MLC). The pedigree included 12,391 animals born from 1974 to 2000 with 329 male and 3,413 female parents. Selection decisions in 1999 were mimicked and potential candidates (1,839) were males born in 1999 and females born between 1996 to 1998 (1 and 3 years generation interval respectively). A total of 1,297 highest EBV ranked animals were included in the optimisations. Optimum contributions were found by using the method described by Grundy et al (1998). Contributions were optimised for both sexes (*opt_all*) and for only male candidates (*opt_males*). In the latter case, female contributions were fixed to *1/2f* where *f* is the number of female candidates. Three pre-defined levels of ΔF were used: 0.005, 0.01 and 0.02.

**Results** The observed ΔF per generation was 0.0046. As the ΔF constraint was less stringent, the number of selected males and females decreased (*opt_all*, Table 1). The regression of contributions on EBVs was significant ($p<0.01$) for all constraints and increased as the restriction on ΔF was less severe (i.e. since the algorithm allocated more unequal contributions to selected animals). Therefore, at the observed ΔF (i.e. 0.005) similar contributions were allocated to selected candidates, whereas when the constraint was relaxed (ΔF=0.02) higher contributions (i.e. more offspring) were assigned to candidates with the highest EBVs. The expected increase in Index EBV compared with the observed value in 2000 (239.4 units) as a result of using the optimisation tool (*opt_all*) ranged from 19.4% (i.e. 285.9 units) for ΔF =0.005, to 27.4% for ΔF =0.02 (Figure 1). The number of selected males to achieve these increases (Table 1) was around the observed number (30), but the number of females was very low compared to about 700 females currently selected per year. This implies unrealistic reproductive rates in females. When fixing female contributions, the number of selected males was reduced substantially (*opt_males*, Table 1), but there was still a substantial expected increase in the Index EBV (from 17.6% for ΔF=0.005 to 19.6% for ΔF=0.02, see Figure 1).

**Table 1** Selected males and females and regression of contributions on EBV ($b_{c,EBV}$, *opt_all*) for three constraints on ΔF.

| ***Optimisation*** | ***DF*** | | |
|---|---|---|---|
| ***opt_all*** | ***0.005*** | ***0.01*** | ***0.02*** |
| Males | 31 | 26 | 18 |
| Females | 49 | 45 | 37 |
| $b_{c,EBV}$ | $1.1x10^{-4}$ | $1.7x10^{-4}$ | $3.4x10^{-4}$ |
| ***Opt_ males*** | ***0.005*** | ***0.01*** | ***0.02*** |
| Males | 12 | 8 | 8 |
| Females | 902 | 902 | 902 |

**Figure 1** Percentage increase in Index EBV after optimisation, relative to observed EBV.




**Conclusions** Selection decisions in the Meatlinc sheep population can be effectively optimised for maximising gain while restricting ΔF by using the dynamic tool. At the observed ΔF, increases of at least 17% in the average Index EBV are expected. Thus, this tool constitutes a potentially highly effective way of managing gain and inbreeding in small livestock populations like the Meatlinc. A smooth integration of this tool in the current structure of sheep Sire Reference Schemes is envisaged.

**Acknowledgments** Work funded by MLC through a LINK SLP Project. Roslin Institute receives support from DEFRA and BBSRC. M.Coffey is acknowledged for providing computing support.

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# Growth and carcass characteristics of crossbred (Mule) sheep

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**Introduction** With increasing emphasis in the meat sector on better and more consistent quality, carcass leanness and conformation is now an important issue for sheep breeders. In 1999, only 47% of all carcasses in the UK met the target specifications for weight, fat and conformation (MLC, 2000), highlighting the potential for improvement. In the current stratified crossbreeding system, crossbred wether lambs are a by-product of the production of dam line ewes for the lowland sector. If their carcass quality is sufficient, they can give a valuable boost to the economics of the breeding programme. Genetic improvement of carcass quality in crossing sire breeds would benefit the crossbred wethers, as well as filter through to the terminal sire cross lambs produced by the crossbred ewes. This work aims to assess the influence of selection index and live conformation score of crossing sires (in this case Bluefaced Leicesters) on growth and carcass quality traits of their crossbred progeny, as a first step towards designing a genetic improvement programme for crossing sire sheep.

**Materials and methods** In each of three years (1998-2000), 1500 hill ewes (equal numbers of Scottish Blackface and Hardy Speckled Face) were mated to 15 Bluefaced Leicester rams from the Penglas Bluefaced Leicester Group Breeding Scheme, selected to represent the full spectrum of index and gigot conformation scores using an elliptical design (Cameron and Thompson, 1986). The index aimed to increase lean content while restricting increases in fat and keeping live weight at 150 days unchanged. In total, 2193 wether lambs were reared and slaughtered when reaching the fat class 2/3L boundary. Live weights were taken at birth, 5, 10 and 16 (weaning) weeks of age and at finished condition, when ultrasonic fat and muscle depth, condition score and live conformation scores (1 improving to 6) were also recorded. Hot and cold carcass weights, MLC fat and conformation scores and conformation on a 15-point scale were taken in the abattoir. A representative sample of 794 carcasses was dissected (80% shoulder joint only, 20% full side dissection). Joints were separated into lean, fat and bone content. The variables were regressed on the index and residual conformation score of the sire. The model included factors for year-of-birth, dam breed, site (3 farms), birth/rearing type (6 classes), age of the rearing dam (4 classes) and the interaction between year and site. The 5-, 10- and 16-week measurements were adjusted to a common age at recording and the finish and slaughter measurements were adjusted to a common condition score.

**Results** Animals weighed on average 27.6 (±4.8) kg at weaning and 35.0 (±4.8) kg at finished condition, giving mean hot and cold carcass weights of 16.2 (±2.2) and 15.8 (±2.1) kg, respectively. The regression on sire's index score was small but positive for most live and carcass weights, positive for ultrasonic muscle depth on the live animal and most muscle measurements on the carcass, and negative for ultrasonic fat depth and carcass fat measurements, in keeping with the design of the index. The regression on sire's conformation score was negative for weight and age of finishing, positive for ultrasonic fat depth and non-significant for any carcass composition measurement. Neither regression was significant for the conformation scores in the live animal or carcass. Some of these results are shown in Table 1.

**Table 1** Unadjusted means (standard deviation in brackets), regression on sire's index score and residual conformation score and standard errors of the regression coefficients for measurements on the live animal and on the carcass

| | | Regr. on index score | | Regr. on conf. score | |
|---|---|---|---|---|---|
| Trait | Raw mean (s.d.) | Regr. coeff. | s.e. | Regr. coeff. | s.e. |
| Ultrasonic fat depth (3d lumbar) [mm]† | 3.6 (1.1) | -0.0013*** | 0.0005 | 0.032* | 0.021 |
| Ultrasonic muscle depth (3d lumbar) [mm]† | 22.1 (2.3) | 0.0060** | 0.0010 | -0.074 | 0.045 |
| Finish weight [kg]† | 35.0 (4.8) | 0.0047* | 0.0022 | -0.360** | 0.095 |
| Age at finish [days] | 186 (63) | 0.022 | 0.028 | -4.52 *** | 1.24 |
| % lean in shoulder‡ | 59.2 (2.9) | 0.0142*** | 0.0022 | -0.009 | 0.101 |
| % fat in shoulder‡ | 23.2 (3.6) | -0.0171*** | 0.0028 | 0.046 | 0.126 |

† measured on live animal; ‡ measured on carcass

**Conclusions** The results show that by using high-index rams the leanness of the carcass of their crossbred offspring can be improved, with no negative effect on live or carcass conformation and with only a small increase in size. The goal to keep the size of crossbred ewes unchanged is considered important to overall efficiency. High-conformation rams gave slightly quicker finishing lambs with larger fat depths, but live and carcass conformation did not improve. This implies selection on index improves carcass quality, whereas the benefit of selection on live conformation remains unclear.

**Acknowledgements** The authors would like to thank DEFRA and MLC for financial support, and the Penglas Bluefaced Leicester Group Breeding Scheme members for the loan of rams.

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# Effects of B vitamins and methyl group donors on milk production, milk composition and blood biochemistry in dairy cows

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**Introduction** Recent reviews highlight the importance of the liver in the coordination of nutrient fluxes in support of pregnancy and lactation (e.g. Drackley *et al*., 2001). Mobilisation of body fat reserves in the late dry period and early lactation leads to an increase in uptake of non-esterified fatty acids (NEFA) by the liver. Their metabolic fate is either oxidation or esterification into triacylglycerides (TAG) that are either exported in very low density lipoproteins (VLDL) or accumulated within liver cells. Recent evidence indicates that TAG accumulation impairs ureagenic and gluconeogenic capacity of the liver, with consequent reductions in feed intake and milk yield, increased incidence of disease and decreased reproductive performance (Overton and Piepenbrink, 1999).

LiFT™ (NuTec Ltd.) is a proprietary mixture of B-group vitamins and methyl group donors (rumen protected choline, niacin, vitamin B12, biotin, folic acid and thiamine) designed to reduce the accumulation of TAG in the liver and accelerate VLDL export. The objective of this experiment was to evaluate the effect of LiFT on milk yield and composition and concentrations of metabolites in blood.

**Material and Methods** Forty non-lactating, pregnant dairy cows were paired according to previous lactation yield and condition score and assigned at random to two treatment groups (Control and LiFT) 3 weeks before expected calving date. Control cows were fed 1kg/d of a standard dry cow compound feed (Pye Farm Feeds, Lancaster). LiFT cows received the same compound feed supplemented with 50kg/t of LiFT. Straw, grass silage and water were available *ad libitum* to both groups of animals. At calving cows were moved to cubicle housing and integrated with the rest of the herd. All cows were fed a semi-complete ration (grass silage, maize silage, barley, Soypro 40 (protein blend, Pye Farm Feeds, Lancaster) and minerals, formulated to support a milk yield of 25kg/d. In addition, cows received a commercial compound feed in the milking parlour (Premium Winter 20, Pye Farm Feeds, Lancaster). The LiFT group received the same compound containing LiFT at 20kg/t. Compound feed was allocated at a fixed rate within each pair and average feed rate across each treatment group was 7kg/cow. Therefore, LiFT cows received an average of 50g LiFT/d in the last three weeks of the dry period and 140g LiFT/d after calving. All cows were fed the control diet from 90d of lactation. Milk yield was recorded daily. Milk crude protein, fat and urea concentrations were measured fortnightly. Venous blood samples were collected from six pairs of cows at an average of 28 and 65 days of lactation.

**Results** Average milk yield during the first 90d of lactation was increased by 3.7kg/d by LiFT (Table 1). This was not associated with any effect on milk composition or blood metabolite concentration.

**Table 1.** *Effect of LiFT on milk yield, composition and somatic cell count (means, 0-90d of lactation)*

| | Control | LiFT | s.e. | *P* |
|---|---|---|---|---|
| Milk yield (kg/d) | 31.7 | 35.4 | 1.02 | 0.03 |
| Milk fat (g/kg) | 40.2 | 39.1 | 0.58 | 0.35 |
| Milk protein (g/kg) | 33.4 | 33.3 | 0.37 | 0.88 |
| Milk urea (mmol/l) | 5.06 | 4.97 | 0.095 | 0.56 |
| Somatic cell count (1000 cells/ml) | 266 | 159 | 60.8 | 0.42 |

**Table 2.** *Effect of LiFT on blood biochemistry (mean of samples collected at 28 and 65d of lactation)*

| | Control | LiFT | s.e. | *P* |
|---|---|---|---|---|
| NEFA (mmol/l) | 0.33 | 0.22 | 0.035 | 0.19 |
| BOHB* (mmol/l) | 0.55 | 0.66 | 0.029 | 0.13 |
| TAG (mmol/l) | 0.11 | 0.10 | 0.004 | 0.88 |
| Bile acid (umol/l) | 75.5 | 56.0 | 4.82 | 0.12 |
| Glucose (mmol/l) | 3.3 | 3.2 | 0.03 | 0.33 |
| Urea (mmol/l) | 5.64 | 5.85 | 0.179 | 0.63 |

*BOHB = betahydroxybutyrate

**Conclusions** LiFT significantly increased milk yield when fed over the last 21 days of the dry period and the first 90 days of lactation, without affecting milk composition or blood metabolites. It is suggested that this effect is due to improved liver function. However, in the absence of data for dry matter intake, or more direct measures of liver function, the mode of action of LiFT cannot be elucidated further. Results demonstrate the benefits of inclusion of a hepatoprotector in the diet of the modern dairy cow.

**Acknowledgement** We are grateful to The University of Liverpool, Department of Veterinary Science for analysis of blood samples.

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# Response of early lactation dairy cows to the inclusion of the liver technology product ABN-LiFT in the total mixed ration

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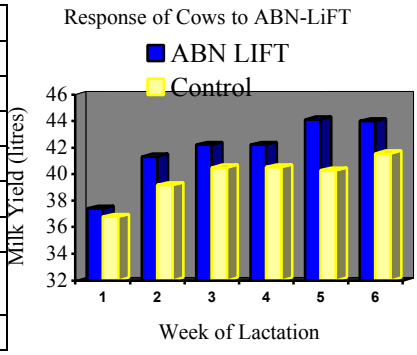
**Introduction.** Through the dry period to early lactation the cow goes through a dramatic change in her metabolism. To supply the high energy requirement in early lactation fat supply from the diet and body mobilisation increases substantially. As a result, the liver accumulates fat, the rate of detoxification slows down, ammonia accumulates and there's a reduced supply of fat, glucose and protein to the udder. This trial was designed to evaluate the response of dairy cows to product called ABN-LiFT a proprietary mixture of B-group vitamins and methyl group donors (rumen protected choline, niacin, vitamin B12, biotin, folic acid and thiamine) designed to reduce the accumulation of triglycerides in the
liver and accelerate VLDL export.

**Materials and Methods.** 48 multiparous cows, housed in cubicles, were used in the two experiments. 32 freshly calved cows were balanced according to calving date, milk yield and somatic cell count into two groups of 16. The remaining 16 dry cows were balanced, according to parity and previous milk yield, into 2 groups of 8. The lactating cows were fed the same total mixed ration plus either the ABN-LiFT or placebo at 100 g per head per day. The pre-calving cows were fed 2 kg of a dry cow roll plus 50 g of the ABN LiFT or the placebo. All cows had *ad-libitum* access to feed, water and cubicles at all times. Milk yields were recorded on a daily basis, with samples taken fortnightly and analysed for fat, protein, lactose and somatic cell count. Cow body condition scored and feed intake were measured weekly. Blood samples were taken 10-20 days pre and post-calving, bloods and analysed by Dairy Herd Health Productivity Service (University of Edinburgh) for β-hydroxybutyrate, glucose, non-essential fatty acids, urea, albumin, globulin and minerals. All data was analysed using Minitab version 13.1.

**Results**. The results shown in the table below are daily averages for cows fed ABN-LiFT in the dry and early lactation plus the lactation period. The figure illustrates the greater response when ABN-LiFT is fed during the dry and early lactation periods.

The results show that weekly milk yields were significantly different between the two groups ($p < 0.001$) with those fed

| | ABN-LiFT | Control | s.e.d. | Signif. |
|---|---|---|---|---|
| Milk Yield (l) | 40.54 | 38.87 | 0.142 | *** |
| Butterfat (%) | 3.99 | 4.09 | 0.061 | NS |
| Milk Protein (%) | 3.36 | 3.09 | 0.298 | NS |
| Lactose (%) | 4.67 | 4.67 | 0.023 | NS |
| Somatic cell count ('000) | 65.2 | 85.1 | 30.2 | NS |
| Body condition score | 2.31 | 2.21 | 0.066 | NS |
| Feed intakes (kg/cow/day) | 53.1 | 50.67 | 1.333 | NS |
| Blood urea N (mmol/l) | 2.83 | 2.41 | 0.167 | * |




ABN-LiFT yielding on average 1.67 litres more than group B (control). Blood urea measurements showed significantly higher ($p < 0.05$) in those cows fed the ABN-LiFT.

**Conclusion**. 'The results suggest that the supply of one or more B vitamins and co-factors to the liver may be limiting the exploitation of the potential of the modern dairy cow. The possible postulated is supported by the higher blood urea that is also indicative of an improved liver function.

**Acknowledgements**. Newton Rigg are grateful for financial funding received from ABNA Ltd.

# The effects of feeding propylene glycol to ewes during late pregnancy and early lactation

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**Introduction** The pregnant uterus has a requirement for glucose that rises rapidly towards the end of pregnancy (Robinson *et al*., 1977). Despite this, the dry matter (DM) intake of the ewe is often depressed during this period causing excessive mobilisation of adipose tissue and an increased concentration of plasma ketones. Propylene glycol resists fermentation in the rumen and following absorption is converted to glucose and glycogen (Andrews, 1982). There is little data available on how in feed inclusion of propylene glycol affects the productivity and energy metabolism of late pregnant and lactating ewes.

**Materials and methods** At 103 days of gestation, 24 twin-bearing Charollais x Lleyn, Charollais x Cambridge, Friesland x Lleyn and Suffolk x Mule ewes were randomly allocated to one of two dietary treatments by breed, age, weight and condition score (CS). All ewes were in lamb to Charollais rams. At 6 weeks *pre partum,* all ewes were individually penned and were fed a basal concentrate (12.2 MJ ME/kgDM; 173 gCP/kgDM) at the rate of 0.6 kg at -6 weeks increasing to 1.1 kg at lambing and 1.6 kg from lambing to +4 weeks. Ewes fed a control diet (C) received the basal concentrate, whilst ewes fed diet PG also received 50 g/d of Energiser 65 Dry (Frank Wright Ltd, Blenheim House, Ashbourne, Derbyshire. UK), equivalent to 32.5 g/d of propylene glycol divided equally between concentrate meals. Winter barley straw was offered to all ewes *ad libitum* and intake was monitored from 3 weeks *pre partum* to lambing. During this period straw refusals were recorded and fresh offered as a single feed at 0800 h at proportionally 1.25 of intake. Ewe liveweight was recorded and CS was measured at weekly intervals. Lamb weight was recorded at birth and at 7, 14, 21 and 28 days *post partum.* Weekly blood samples were analysed for ß-hydroxybutyrate (BHB), non-esterified fatty acids (NEFA) and glucose. The data was analysed using a completely randomised block design and subjected to analysis of variance.

**Results** There was no effect of dietary treatment on the intake of concentrate, straw, total DM, total ME or on total CP (Table 1). There was also no effect of diet on the weight or CS of ewes at 6 weeks *pre partum* (74.5 *v*. 72.7 kg) 1 week *pre partum*, immediately *post partum* or at 4 weeks *post partum*. In addition, there was no effect of diet on either the *pre partum* or the *post partum* weight or CS change of ewes and there was no effect on the lamb birth weight, lamb growth rate or lamb weight at 21 days of age. However, ewes fed diet PG had a higher concentration of plasma glucose at 4 weeks *pre partum* than ewes fed diet C (2.78 *v*. 2.37; $P<0.01$; Table 2). The concentration of plasma BHB tended to be lower in ewes fed diet PG compared to those fed diet C at 4 weeks *pre partum* (0.39 *v*. 0.46 mmol/l; $P=0.065$) and at 2 weeks *pre partum* (0.45 *v*. 0.59 mmol/l; $P=0.056$) and was significantly lower during the second week of lactation (0.55 *v*. 0.84 mmol/l; $P<0.01$). The concentration of plasma NEFA was lower in ewes fed PG at 4 (0.29 *v*. 0.39; $P<0.001$) and at 2 (0.31 *v*. 0.42; $P<0.01$) weeks *pre partum.*

**Table 1** *Nutrient intake and ewe performance*

| | C | PG | s.e.d. | Sig. |
|---|---|---|---|---|
| Straw intake *(kg/d)* | 0.50 | 0.51 | 0.047 | NS |
| Total DM intake *(kg/d)* | 1.25 | 1.26 | 0.047 | NS |
| Total ME intake *(MJ/d)* | 12.39 | 12.45 | 0.307 | NS |
| Total CP intake *(g/d)* | 154 | 154 | 2.3 | NS |
| *Pre partum* ewe CS change | -0.19 | -0.13 | 0.131 | NS |
| *Post partum* ewe CS change | -0.73 | -0.88 | 0.175 | NS |
| *Pre partum* ewe wt change *(kg)* | +7.8 | +9.3 | 0.93 | NS |
| *Post partum* ewe wt change *(kg)* | -5.0 | -6.0 | 1.83 | NS |
| Lamb birth weight *(kg)* | 3.87 | 4.08 | 0.227 | NS |
| Lamb weight at 21 days *(kg)* | 8.39 | 9.26 | 0.512 | NS |
| Lamb growth rate *(g/d)* | 238 | 247 | 12.3 | NS |

**Table 2** *Blood metabolite concentrations*

| | C | PG | s.e.d. | Sig. |
|---|---|---|---|---|
| Plasma glucose *(mmol/l)* | | | | |
| -4 | 2.50 | 2.78 | 0.072 | ** |
| -2 | 2.63 | 2.75 | 0.108 | NS |
| +2 | 3.38 | 3.31 | 0.204 | NS |
| Plasma BHB *(mmol/l)* | | | | |
| -4 | 0.46 | 0.39 | 0.036 | NS |
| -2 | 0.59 | 0.45 | 0.065 | NS |
| +2 | 0.84 | 0.55 | 0.078 | ** |
| Plasma NEFA *(mmol/l)* | | | | |
| -4 | 0.39 | 0.29 | 0.021 | *** |
| -2 | 0.42 | 0.31 | 0.035 | ** |
| +2 | 0.61 | 0.65 | 0.076 | NS |

**Conclusions** In the current experiment, ewes were housed indoors and individually fed at a level close to their ME requirements. Despite this, feeding ewes a diet containing 32.5 g/d of propylene glycol in the concentrate was effective at increasing plasma glucose concentration and reducing plasma BHB and NEFA concentrations. Feeding propylene glycol to ewes that are experiencing a more severe energy deficit than that described in the current experiment could be an appropriate method of reducing the incidence of pregnancy toxaemia.

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# The effect locomotion score and lameness and on dry matter intake and behaviour in dairy cattle

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**Introduction** Lameness has been identified as an extremely painful condition (Manson and Leaver, 1988). Studies have found increased locomotion score (LS) and lameness to reduce productivity, while other have found no such reduction (Manson and Leaver, 1988; Kelley et al., 1990; Phillips et al., 1994). Changes in time spent feeding have been associated with changes in LS (Manson and Leaver, 1988) and less time lying down (Hassall, 1993). However, while lame cows change their feeding and general behaviour there is little information regarding the extent and mode of these changes. The objective of this study was to measure the effect of locomotion score on behavior and feed intake.

**Materials and methods** A total of 165 dairy cows were observed over a three-year period on the University dairy farm. The observations took place in the autumn of 1998, 1999 and 2000. The cows were observed for locomotion score on a 5 point scale (1 = non lame, 5 = very lame) according to (Manson and Leaver, 1988). The locomotion score (LS) groups were balanced for live weight, condition score and previous milk yield. All LS groups were offered the same completed mixed ration in each year from an electronic and computerized feeding system, which identified each cow individually and recorded the time of feed, duration of the meal, size (kg) of meal and the total number of meals per cows per day. In addition general behaviour of the cows was monitored 24 hours a day using time lapse video equipment. The time spent in cubicles, passage and loafing areas and standing, lying times were recorded. The experiment was a randomised block design, lame cows were paired with non lame herd mates who had calved during the same time period and balanced for the effect of previous lactation milk yield, live weight and condition score. The normally distributed data was analysed by ANOVA (Minitab), significant differences between means were tested by t test.

**Results**

**Table 1.** Dry matter intake, meal number, duration, size and time spent in cubicles by cows with varying locomotion scores (1 = non lame, 5 = very lame)

| | Locomotion score | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | Sem | Sig. |
| Observations (animals) | 30 | 30 | 30 | 30 | 15 | | |
| Dry matter intake (kg/d) | 28.41 [a] | 26.01 [a] | 25.01 [a] | 24.51 [a] | 24.0 [b] | 0.83 | ** |
| Number of meals (/24 h) | 38.1 [a] | 29.6 [a] | 21.1 [b] | 15.1 [b] | 20.0 [b] | 3.20 | ** |
| Mean meal size (kg DM) | 1.5 [c] | 2.0 [bc] | 2.2 [ab] | 2.5 [ab] | 3.0 [a] | 0.40 | ** |
| Time in cubicles (min) | 280.0 [b] | 275.2 [b] | 320.4 [ab] | 400.1 [a] | 408.2 [a] | 31.0 | ** |

NS - Mean values in rows did not differ significantly P>0.05.
[a b c] ** Data in rows followed by differing subscripts differ significantly (P< 0.05)

At locomotion score of 5 very lame cows had significantly lower dry matter intakes compared with non-lame cows. Increasing locomotion score significantly affected the feeding pattern of dairy cattle and lame cows with a locomotion score of 4 and 5 consumed a significantly lower number of meals per day, with significantly longer meal duration per meal and greater meal size compared with non- lame cows with locomotion scores of 1 and 2.

**Conclusions**
Up to a locomotion score of 4, cows are capable maintaining the level of dry matter intake by adjusting their feeding behaviour. However, very lame cows with a locomotion score of 5 have lower dry matter intakes. Lame cows (LS 4 and 5) would benefit from preferential feeding management in order to maintain dry matter intake levels.

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# The effect of biotin supplementation on the mechanical properties of the hoof horn and lameness in dairy cows

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**Introduction** Biotin plays a critical role in the differentiation of epidermal tissue, in the fatty acid and glucose metabolism. Qualitative and quantitative changes in the synthesis of keratin filaments, interruption of the co-ordination from keratinisation until cornification and intercellular cementing substance of poor quality occur in the hoof horn of biotin deficient calves (Mulling et al., 1999). The addition of supplementary biotin in diets has been found to significantly increase hoof hardness and reduce lameness of cows (Schmid, 1995; Fitzgerald et al., 2000). The aim of this experiment was to compare changes in locomotion score and lesion score with the results of mechanical testing of the sole and white line horn in cows supplemented and not supplemented with biotin.

**Materials and methods** The influence of biotin on mechanical properties of the hoof horn was investigated in 18 cows supplemented with biotin and 18 non-supplemented. After calving, the cows received a lactating cow diet consisting of grass and maize silage (50:50 ratio). In addition, cows received a maximum of 8 kg of concentrated feed (22 % CP) in the parlour and 2 kg through out of parlour feeders (OPF). The concentrate offered through OPF for the biotin group had an additional 22 mg biotin / cow/ day, the control group had 2 kg with no added biotin. The cows were loose housed in an area consisting of 42 cubicles fitted with cow mats and sawdust. All cows in the experiment were scored for lameness twice weekly. The lesions on each foot were scored according to Leach et al. (1998) at 40, 100 and 150 days *post partum.* At the same time samples of hoof sole tissue were collected from all claws. Samples were kept in sealed plastic bags and stored in a refrigerator at a temperature of 2 °C until analysed on a texture analyser. Samples were analysed for puncture resistance (Pforce) and elastic-modulus. The experiment was a randomised block design, using individual cows as observations. All the parameters were compared by treatment, collection periods and claws, using ANOVA, general linear modelling command (Minitab 12.0). The comparison of means of the periods and the claws was completed using the Tukey test.

**Results** There were no significant differences in puncture resistance of the sole and white line horn, the difference of the puncture resistance between hooves collected at day 150 and 40 of lactation, elastic modulus, lesion score and locomotion score between biotin supplemented and not supplemented cows (Table1). However, biotin supplemented cows presented better values in most parameters than non biotin supplemented cows. There was a significant decrease in puncture resistance of the sole and white line horn ($p<0.001$) and increase in the lesion score of all claws ($p<0.05$) between day 40 of lactation and days 100 and 150. Puncture resistance of the sole and the white line horn of the front claws were significantly greater than the hind claws and the lesion score was lower ($p<0.001$). The dry matter of the sole horn was significantly ($p<0.01$) correlated to puncture force of the sole horn in day 100 and 150 of lactation.

**Table 1** *Pforce of sole and white line (WL), difference in Pforce between days 150 and 40, elastic modulus, loc. score and lesion score on day 150 of biotin supplemented and not supplemented cows*

| parameters | biotin | control | sem | *p* |
|---|---|---|---|---|
| Pforce sole (N) | 802.5 | 817.4 | 18.8 | ns |
| Pforce WL (N) | 536.4 | 587.6 | 20.7 | ns |
| Dif Pforce sole days 150 and 40 (N) | -20.6 | -74.3 | 33.1 | ns |
| Dif Pforce WL days 150 and 40 (N) | -80.5 | -121.7 | 44.0 | ns |
| Elastic modulus ($N/mm^2$) | 120.4 | 107.9 | 16.3 | ns |
| Lesion score sole on day 150 | 1616.0 | 1739.1 | 80.0 | ns |
| Lesion score WL on day 150 | 1673.0 | 1751.5 | 95.2 | ns |
| Locomotion score, mean (1-5) | 1.82 | 2.04 | 0.21 | ns |

**Conclusions** The lack of significant difference in lesion score and mechanical properties between biotin supplemented and non supplemented cows was reported by Midla et al. (1998) and Schmid (1995). This lack of significant difference is related to the large variation of the measurements between cows and indicates the need to work with a bigger number of animals. Mechanical properties were related to lesion scores at different periods and to the locomotion score indicating that these measurements are a good indicator of hoof horn quality.

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# Effects of body condition and dry cow feeding on disease incidences in the first 100 days of lactation in Holstein-Friesian cows

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**Introduction** Genetic potential for milk production has increased rapidly in the Holstein-Friesian breed and there is concern that this might be causing an increased incidence of health problems. We have recently (Ingvartsen *et al*., 2002) reviewed the inter-relationships between lactation performance and health, demonstrating the importance of considering effects on/of body reserves as well as effects on/of milk production. Whilst we identified mechanisms whereby body reserves can have a direct effect on susceptibility to disease, disease also affects body reserves making it difficult to study their inter-relationships. The dry period is a particularly interesting period in this regard, because additional nutrients are directed towards reserves, whilst it is followed by a period (early lactation) of high disease incidence. The objective of this study was to investigate the effects of body condition score (BCS) at calving, as well as the effects of dry period diets designed to alter BCS, on disease incidences in the first 100 days of lactation.

**Material and methods** This analysis used information from 164 Holstein-Friesian cows involved in 4 dry cow feeding experiments conducted over the period 1994 to 1999. Dry cow treatments were as follows: Expt. 1: grass silage *ad libitum* (GS); GS with 0.5 kg/day prairie meal (GSPM); 60/40 (DM basis) mixture of grass silage and barley straw *ad libitum* (GSBS); Expt. 2: GS; GSPM; GSBS; GSBS with 0.5 kg/day prairie meal; Expt. 3: GS; GSBS; GS with 2 kg/d of concentrates (CP=237 g/kg DM) for the final 3 weeks; GSBS with 2 kg/d of concentrates for the final 3 weeks; Expt. 4: GS, GSPM. Body condition score was recorded (0 = emaciated; 5 = obese) in the week prior to calving. After calving, all cows received the same diet, within each experiment, based on grass silage *ad libitum* and a flat-rate allocation of concentrates (10, 6, 8 and 8 kg/day for the 4 experiments respectively). Cases of lameness, metritis and mastitis were recorded over the first 100 days of lactation and coded (0 = absence; 1 = presence) for each cow. Results were analysed using a Generalised Linear Model, with a binomial distribution and canonical link function (Genstat 5; Lawes Agricultural Trust, 1998) and the following terms: Experiment + Parity + BCS + Dry Period Feeding Level. Dry period diets were categorised into 3 levels (0 = GSBS; 1 = GS or GSBS with supplements; 2 = GS with supplements).

**Results** The mean proportions of cows with each disorder in the first 100 days of lactation were 0.207, 0.219 and 0.195 for lameness, metritis and mastitis respectively. Parity averaged 3.8 (SD = 1.88; range = 2 to 10) and pre-partum BCS averaged 2.4 (SD = 0.45; range = 1.25 to 4). Pre-partum BCS was significantly affected by dry period feeding level (2.26, 2.44 and 2.60; SED = 0.087; $P<0.01$ for the 3 feeding levels respectively).

**Table** *Estimated effects of parity, pre-partum BCS and dry period feeding level on the incidence of lameness, metritis and mastitis in the first 100 days of lactation. SE are shown in parentheses.*

| | Parity | Pre-partum BCS | Dry Period Feeding Level |
|---|---|---|---|
| Lameness | +0.22 (0.111)$^{*}$ | -1.53 (0.582)$^{**}$ | +0.95 (0.317)$^{**}$ |
| Metritis | +0.42 (0.114)$^{***}$ | -0.46 (0.529)$^{NS}$ | -0.55 (0.304)$^{(P = 0.07)}$ |
| Mastitis | +0.15 (0.107)$^{NS}$ | +0.34 (0.512)$^{NS}$ | +0.37 (0.296)$^{NS}$ |

**Conclusions** As expected, there were increased incidences of these disorders in older cows: the lack of significant effect on mastitis may be related to early culling of cows on the basis of mastitis. There were apparently contradictory effects of BCS and dry period feeding level on the incidence of lameness, highlighting the difficulties of studying the relationships between diet, lactation performance and health. Increased feeding level in the dry period, which would lead to an increase in BCS, led to increased lameness. However, there was also significantly increased lameness in thinner cows, which may reflect a curvilinear effect of BCS (which we could not adequately test) or which may be an effect rather than a cause (i.e. cows that are repeatedly lame lose body condition). Effects of BCS and dry period feeding level on the incidences of metritis and mastitis failed to attain significance at the 5% confidence level, probably because of the limited sample size, but were consistent. Increasing the level of feeding in the dry period tended to reduce the incidence of metritis.

**Acknowledgements** The financial support of DEFRA, the Milk Development Council and the Danish Ministry of Food, Agriculture and Fisheries is gratefully acknowledged.

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# Influence of Genetic Merit on Mastitis and Lameness in Dairy Cattle on Commercial Farms

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**Introduction** The cost of disease within the dairy industry is associated with loss of yield, discarded milk, cost of treatment and reduced fertility. Mastitis and lameness are two of the most financially important health problems associated with dairy production in the UK. Mastitis alone is estimated to account for approximately 10% of all cull cows and to have an associated cost of £218 per case (Kossaibati and Esslemont, 1995). Lameness accounts for approximately 6% of cull cows and has an associated cost of £273 per case (Kossaibati and Esslemont, 1995). In the present study somatic cell count (SCC) and locomotion score (LS) records were used as indicators to estimate the influence of genetic merit for milk production on mastitis and lameness.

**Materials and Methods** 5967 and 8236 monthly test day records of somatic cell counts and locomotion scores (recorded from commercial herds in Kent), respectively, were used to estimate the influence of genetic merit (PIN95) on health in dairy cattle on commercial farms. Model 1 was the final model used for somatic cell count data, and Model 2 for locomotion score data. Both somatic cell count and locomotion score data were transformed to the logarithm$_{10}$ to ensure a normal distribution. Model 1 explained 13% of the variation in Log10(SCC), and Model 2, 9% of the variation in Log10(LS). Housing system was used to describe herd environment.

Model 1 Log10(SCC) = YOC + SOC + MOR + P + S + H + C + P*H + C*H + G*H

Model 2 Log10(LS) = YOC + MOC + MOR + P + S + H + G + C + G*H

Where Log10(SCC) was the natural logarithm of somatic cell counts and Log10(LS) was the natural logarithm of locomotion scores; YOC was year of calving, SOC was season of calving, MOC was month of calving, MOR was month of recording, P was parity, S was stage of lactation, H was housing system, C was concentrate intake (kg/d/cow), G was genetic merit (PIN95) and * indicates an interaction.

**Results** Table 1 shows that somatic cell counts (SCC) were higher for cows housed in straw yards (Log10(SCC) = 2.09) compared with cubicles (Log10(SCC) = 1.85). There was a significant ($p<0.05$) increase in Log10(SCC) with PIN95 in straw yards but not in cubicles (Table 2). Locomotion scores were higher for cows housed in cubicles (Log10(LS) = 0.15) compared with straw yards (Log10(LS) = 0.10) (Table 1), and there was a reduction in Log10(LS) with genetic merit under both housing systems (Table 2). The results indicated that 6 and 3% of the recordings in cubicles and straw yards, respectively, showed cows to be clinically lame (defined as LS ≥ 3).

**Table 1:** Least Square Means, standard errors and geometric means for Log10(SCC) and Log10(LS) in straw yard and cubicle housing systems

| Housing | Log10(SCC) | | | Log10(LS) | | |
|---|---|---|---|---|---|---|
| | Mean | SEM | Geometric Mean | Mean | SEM | Geometric Mean |
| Cubicle | 1.85 | 0.014 | 71.15 | 0.15 | 0.003 | 1.41 |
| Straw Yard | 2.09 | 0.021 | 123.33 | 0.10 | 0.003 | 1.26 |

Where Log10(SCC) is the natural logarithm of somatic cell count and Log10(LS) is the natural logarithm of locomotion score

**Table 2:** Estimates of the influence of PIN95 on Log10(SCC) and Log10(LS) in straw yard and cubicle housing systems

| Housing | Log10(SCC) | | | Log10(LS) | | |
|---|---|---|---|---|---|---|
| | b | SEM | *p-value* | b | SEM | *p-value* |
| Cubicle | -0.0003 | 0.00037 | *0.383* | -0.0004 | 0.00009 | *<0.001* |
| Straw Yard | 0.0011 | 0.00046 | *0.022* | -0.0007 | 0.00011 | *<0.001* |

Where Log10(SCC) is the natural logarithm of somatic cell count and Log10(LS) is the natural logarithm of locomotion score, b is the regression co-efficient for PIN95

**Conclusions** Somatic cell counts increased with genetic merit for straw yard but not for cubicle systems, and there was a higher somatic cell count for straw yard versus cubicle systems. This suggests that there was a higher incidence of mastitis within straw yard systems as indicated by SCC. These findings may relate to the longer lying times that occur in straw yards compared with cubicles, the presence of larger slacker teat sphincters in animals producing higher milk yields (Dodd and Neave, 1951) and higher levels of environmental mastitis causing organisms in straw yards compared with cubicle housing. In contrast to SCC, LS were shown to be lower in straw yard compared with cubicle housing systems which indicates higher levels of lameness within cubicle housing systems. The finding that locomotion score improved with genetic merit for milk production suggests that the selection of cattle for milk production does not increase lameness.

**Acknowledgements** The authors wish to thank the South of England Agricultural Society and Wye College, University of London for financial support, and Philip Drury for help with the collection of data.

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# The effect of molybdenum, iron and sulphur supplementation on immune function in growing lambs

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**Introduction** Clinical copper (Cu) deficiency in ruminant animals is mainly attributed to antagonistic interactions with molybdenum (Mo), iron (Fe) and sulphur (S) and Williams *et al.* (2001) reported differential effects between Mo and S, compared with Fe and S on various copper parameters. Suttle and Jones (1986) reported an increased mortality of lambs and infection rate due to copper deficiency which suggests an effect of Cu deficiency on immune function. The aim of this study was to assess the effects of Mo or Fe and S on the cellular and humoral immune responses of growing lambs.

**Materials and methods** Twenty-four Charollais X Friesland lambs with an initial mean liveweight of 27.3kg (s.e.d 1.38) were group housed and randomly allocated to one of three dietary treatment groups with eight lambs per group (4 male, 4 female). All lambs were fed *ad libitum* a basal complete diet based on straw pellets (405 g/kg), barley (300 g/kg), rapeseed (200 g/kg), molasses (60 g/kg) and mineral premix (35 g/kg) (ME 10.6 MJ/kg DM : CP 138.6 g/kg DM: 3.6 mg/kg Cu) during a fourteen day adaptation period. The control treatment group (Control) continued to receive the basal diet for the duration of the trial, group two (Fe group) received an additional 500mg/kg DM Fe and 2 g/kg DM S and group three (5 Mo) received an additional 5 mg/kg DM Mo and 2 g/kg DM S. During week 4 and 8 of the trial all lambs were immunised with 1 mg of the novel antigen Keyhole Limpet Haemocyanin (KLH) (Calibiochem), precipitated in alum and given subcutaneously at a site above the ribs. Blood samples were collected by jugular venepuncture weekly from week 4 onwards to assess anti-KLH responses. Lamb anti-KLH IgG and IgM responses were measured by direct ELISA at an optical density (OD) of 405nm. Lymphocyte blastogenesis was assessed using a modified method of Mosmann *et al.* (1983) using the mitogens Concanavalin A (Con A) (5µg/ml) and pokeweed mitogen (PWM) (5µg/ml) (Sigma) and the antigen, KLH (25µg/ml). Statistical analysis of the results was by ANOVA.

**Results** Figure 1 indicates that from the first immunisation of KLH (week 4), there was an increase in the primary IgG response, which reached a peak after two weeks. A peak in the secondary response occurred two weeks after the second immunisation (week 8). There was no significant difference between treatments ($p>0.05$) at any timepoint for IgG response. Similarly, there was no significant differences ($p>0.05$) between treatments at each timepoint for IgM response (Fig. 2), although increases in optical density can be seen after KLH was administered after week 4 and 8. There were no significant differences ($p>0.05$) between dietary treatments on lymphocyte blastogenic response of PWM, Con A, KLH or Control during weeks 6,9 and 12.

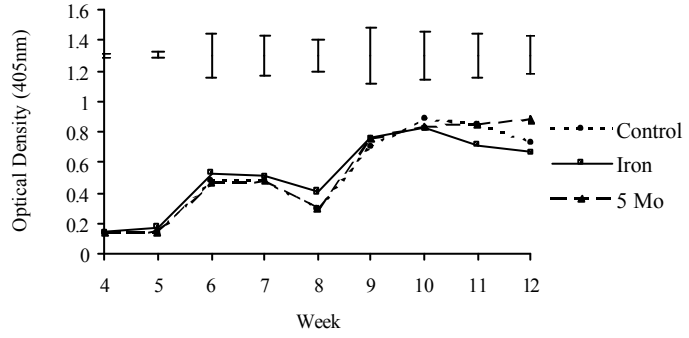


**Figure 1** Effect of dietary treatment on Anti-KLH IgG response

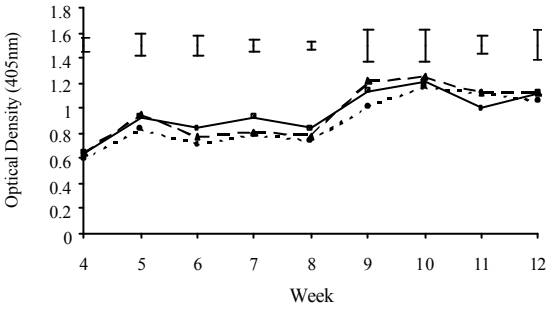


**Figure 2** Effect of dietary treatment on Anti-KLH IgM response

**Conclusion** Previous work has reported that copper deficiency has a detrimental effect on innate neutrophil function in lambs (Suttle and Jones, 1986). However, these finding indicate that there was no effect of antagonistic minerals on specific cellular and immune responses in lambs, similar to findings on immune function in calves (Ward *et al.* 1997).

**Acknowledgement** This work was funded by the Silcock Foundation.

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# Replacement of lucerne hay with bagasse pith treated with *Pleurotus ulmarius* in the diet of finishing Shal lambs

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**Introduction** The treatment of sugarcane pith with various methods in order to upgrade its nutritive value and to increase the utilisation of its energy by ruminants has been widely employed in many parts of the world (Sansoucy *et al.* 1988). The aim of the experiment was to improve the nutritive value of sugarcane pith by a biological treatment and to assess the possibility of its maximum inclusion as a replacement with Lucerne hay in the diet of finishing Shal lambs.

**Materials and methods** *Pleurotus* (*Hypsizygus*) *ulmarius* was grown from cultures provided by Biologische Pilze. The fungus was cultured on sugarcane bagasse pith for a period of 80 days at 20-25 $^{o}$C and 80% humidity in the dark. At the end of fungal growth, the edible fruit bodies were harvested for human food and the spent bagasse substrate was dried in air and used to feed the experimental animals. The chemical composition of the treated and untreated bagasse pith was also measured. Twenty-four three and half months old Shal lambs (mean live weight 26 ± 4.1 kg) were used for a period of twelve weeks in this experiment. The animals were randomly allocated by weight into two groups (A and B) each consisted of three replicates of four lambs in a pen. They were given a diet consisted of concentrate 600 g/kg and roughage 400 g/kg based on NRC requirements. For group A the roughage consisted only of lucerne hay which offered *ad libitum* (defined as the lucerne hay diet - LHD) and for the group B it consisted of half of the lucerne hay used in group A and *ad libitum* amounts of the fungal treated bagasse pith (the TBD diet). The concentrate contained (g/kg) ground barley 500, wheat bran 260, cottonseed meal 220 and mineral/vitamin supplement 20. Live weight gains were measured biweekly and feed intakes was also determined for each group of replicates daily. Data compared between TBD and LHD groups for the whole experimental period by one-way analysis of variance using Minitab.

**Table 1:** Chemical composition of feed ingredients

| | DM | g/kg dry matter | | |
|---|---|---|---|---|
| Ingredients | g/kg | CP | NDF | ADF |
| Untreated BP | 932 | 16 | 853 | 570 |
| Treated BP | 923 | 95 | 705 | 547 |
| Lucerne hay | 935 | 175 | 448 | 379 |
| Barley grain | 911 | 109 | 218 | 99 |
| Wheat bran | 921 | 157 | 490 | 122 |
| Cottonseed meal | 952 | 373 | 354 | 290 |

BP = bagasse pith

**Table 2:** Effects of the treatment diets on animal performance during 12 weeks of the experimental period

| | Treatments | | | |
|---|---|---|---|---|
| Parameters | LHD | TBD | Sem | Sig |
| Initial weight (kg)[a] | 26.1 | 26 | 1.24 | NS |
| Final weight (kg) [a] | 37.4 | 35.1 | 1.32 | NS |
| Daily gain (g) [a] | 134 | 108 | 16.2 | NS |
| DM intake (g/d) [b] | 1155 | 1089 | 17.6 | NS |
| FCR (kg feed/kg gain) [b] | 8.6 | 10.1 | 0.31 | NS |
| LH intake (g/d) [b] | 583± 21 | 224± 35 | | |
| TBP intake (g/d) [b] | 0.0 | 257± 12 | | |

Values are means of twelve animals (a) or three groups of replicates (b). Sem = Standard error of means LH= Lucerne hay TBP = Treated bagasse pith ± = means ± Std.

**Results** The degradation of bagasse pith by *Pleurotus* resulted in a decrease of NDF content from 853 to 705 g/kg and an increase of the protein content from 16 to 95 g/kg on a dry matter basis (Table 1). ADF content of bagasse pith was not changed significantly. No significant differences were observed in the amount of feed intake between the two groups ($P>0.05$). Despite of the availability of bagasse pith to TBD group, the mean consumption of this material was only 257±12 g/d compared to the Lucerne hay consumption of the LHD group which was 583± 21 g/d (Table 2). No significant differences were observed between the two groups in their average daily liveweight gain (ADLWG) and feed conversion ratio (FCR) during the whole period of the experiment (Table 2) but there was a significant ($P<0.05$) decrease in the ADLWG of TBD group during the first two weeks of the experiment. Although the average live weights of the lambs with LDH were always higher than that of lambs with TBD, the difference was not statistically significant ($P>0.05$).

**Conclusion** The results of this study indicate that Lucerne hay could be replaced with bagasse pith treated with *Pleurotus* fungi in the diet of finishing lambs up to 560 g/kg without significantly adverse effects on live weight gain, feed intake and feed conversion ratio. The possibility of the inclusion of this fairly cheap waste product material in the diet of breeding ewes, dairy cattle and beef cattle needs further study and the cost effectiveness assessed.

**Acknowledgement** This work was funded by Iranian research organization for science and technology (IROST). The authors would like to thank staff of Aminabad research institute of veterinary faculty for their help in this study.

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# Performance of weaner pigs when fed diets containing different combinations of exogenous xylanase and betaine

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**Introduction** Exogenous xylanases reduce anti-nutritional factors in feedstuffs and help optimise digestion in the small intestine through their positive effects on water holding capacity, nutrient packaging, digesta viscosity and flow. The net effect is reduced likelihood of both undesirable bacterial proliferation in the small intestine and migration of bacteria from the large to the small intestine leading to a disease challenge and hence reduced performance. Osmotic upsets have also been observed with pigs fed cereal-based diets, and the osmoregulatory role of betaine may strengthen the pig's defence of this condition. As the mode of action of betaine is different from enzymes, it has been postulated that piglets may be responsive to combinations of these additives. The object of the experiment reported was to study the performance of weaner pigs fed diets containing differing rates of inclusion of exogenous xylanase and betaine.

**Material and Methods** Seventy-two male hybrid pigs at weaning (7.3 ±1.0 kg live weight) were individually penned and randomly allocated immediately to one of 12 treatments until they reached 28.7±1.5 kg live weight. Treatments were 1) Control (C); 2) C+1 kg Xylanase product (X)/t ; 3) C+ 2kg Betaine (B2)/t ; 4) C+B2+ 0.25kg X/t ; 5) C+B2+ 0.5kg X/t; 6) C+B2+ 0.75kg X/t ; 7) C+B2+1.0kg X/t ; 8) C+ 1kg Betaine (B1)/t; 9) C+B1+ 0.25kg X/t; 10) C+B1+ 0.5kg X/t; 11) C+B1+ 0.75kg X/t; 12) C+B1+ 1.0 kg X/t. The dry xylanase product had a minimum guaranteed activity of 4000 U/g (Porzyme® 9300) and betaine was a minimum 96% pure, dry product (Betafin® S1). All treatments were offered *ad libitum* as a two phase feeding regime; phase 1 for the first 21 days and phase 2 from day 21 to completion. The diets were multi-ingredient (mainly wheat, barley, cooked wheat and soyabean meal) and contained 9.75 / 9.61 MJ NE/kg and 11.4 / 10.5 g digestible lysine /kg (phases 1 and 2 respectively). The ingredients were coarsely ground (4 mm sieve), mixed and pelleted at 67°C by Roslin Nutrition Ltd. A small (250g) sample of slurry obtained at the same time as the piglets was deposited into each pen. Live weight gain was calculated as the linear slope of the response of live weight (recorded weekly ) to time (days) using GENSTAT 5.3 for Windows. This analysis was conducted over the entire duration of the trial. Solving the linear equations for each piglet for a live weight of 7.5 and 27.5kg allowed a precise estimate of the initial and final day on trial. This then allowed an adjustment to recorded food intake to give the actual amount of food required to grow from 7.5 to 27.5kg. The data were analysed to two ways:1) Four treatments, 1, 2, 3 and 8; and 2) 2*5 factorial (treatments 3 to 12 inclusive) , where responses were analysed by establishing linear and non-linear (quadratic) contrasts with numerical values (0, 25, 50, 75 and 100) declared.

**Results**

**Table 1** Effect of four treatment model on performance of post-weaning pigs over the complete experimental period

| Treatment | Control (1) | Control + 1kg Xylanase/t | Control plus 2kg Betaine/t | Control plus 1kg Betaine/t | s.e.d. | P value |
|---|---|---|---|---|---|---|
| Daily live-weight gain (kg) | 0.542 | 0.577 | 0.560 | 0.591 | 0.031 | NS |
| Food conversion ratio | 1.52 | 1.54 | 1.57 | 1.50 | 0.0612 | NS |
| Total food intake/pig (kg) | 30.5 | 30.7 | 31.4 | 30.0 | 1.22 | NS |

**Table 2** Effect of 2*5 factorial model on performance of post-weaning pigs (betaine treatments amalgamated)

| | Xylanase product added /tonne | | | | | | |
|---|---|---|---|---|---|---|---|
| Treatment | 0 kg Control[1] | 0.25 kg | 0.5 kg | 0.75 kg | 1.0 kg | s.e.d. | P value |
| Daily live-weight gain (kg) | 0.576 | 0.564 | 0.545 | 0.584 | 0.593 | 0.106 | 0.106 |
| Food conversion ratio | 1.53 | 1.75 | 1.56 | 1.50 | 1.48 | 0.122 | NS |
| Total food intake/pig (kg) | 30.7 | 34.9 | 31.1 | 30.0 | 29.6 | 2.43 | NS |

[1] Control treatments included either 1kg or 2kg Betaine added/tonne in factorial model.

No effects of treatment were recorded on faecal consistency and no ill-health was observed .The four treatment model demonstrated no statistically significant effect of either xylanase or betaine supplementation on performance compared with the control treatment. The factorial analysis showed that the highest xylanase product supplementation (1 kg/t) tended to increase performance over the betaine-only supplemented treatments (table 2), resulting in a statistically significant quadratic response for live-weight gain (P = 0.047).

**Conclusions** Although, the numerical differences between the individual supplement treatments and the control were not statistically significant, the combination of xylanase and betaine indicated superior performance, through the quadratic response, when added at levels that were similar to those previously reported by van Lunen and Simmins (2000; 1 and 1.5 kg/t xylanase product and betaine respectively). The data indicated that maximal piglet response may not have been achieved in this experiment and may have required even higher levels of xylanase (Porzyme® 9300).

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# Effect of increasing stearoyl coenzyme A desaturase mRNA concentrations using forage and concentrate diets on the fatty acid composition of ovine adipose tissue

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**Introduction** Compared to meat from other animals lamb contains high levels of saturated fat, particularly stearic acid which comprises 18% of the total fatty acids (Enser *et al*, 1996). This stearic acid can be desaturated in the tissue by stearoyl coenzyme A desaturase (SCD) to produce oleic acid. In sheep SCD is produced from a single gene and the levels of SCD mRNA in the tissue correlate well with oleic acid (Ward *et al*, 1998, Barber *et al*, 2000) suggesting that an upregulation of SCD activity may increase the relative proportions of unsaturated and saturated fatty acids and so significantly improve the nutritional quality of sheep meat. Our recent studies have shown that insulin increases SCD mRNA levels and monounsaturated fatty acid synthesis in cultured ovine adipose tissue explants (Daniel *et al*, 2001). The present study was designed to investigate whether feeding a diet believed to manipulate SCD mRNA concentrations would significantly alter the fatty acid composition of lamb.

**Materials and Methods** Twenty four Mule x Charolais ewe lambs approximately 8 weeks old (average initial liveweight 28.6 kg) were randomly allocated to one of 3 treatment groups: grass nuts (diet 1), restricted barley-oats based concentrate diet to give the same growth rate as the grass nuts (diet 2), or the same concentrate diet approaching *ad libitum* intake (diet 3). The trial was conducted over a 7-week period. At slaughter, samples of subcutaneous (rump), omental and perirenal adipose tissue were taken and snap frozen in liquid nitrogen. SCD mRNA levels were determined using a ribonuclease protection assay. Lipid was extracted using a 2:1 chloroform to methanol solution and the samples were prepared using a base methylation and analysed by GC using a 60m BPX70 column. Statistical analyses were performed using a split-plot two-way ANOVA to compare the effects of diet between sheep and the differences between depots within sheep.

**Results** The animals fed grass nuts grew at 180 g/d, animals fed restricted concentrate grew at 178 g/d and animals fed *ad-libitum* concentrate grew at 260 g/d. There was no significant interaction between diet and depot in any of the parameters measured. In all depots the animals fed diet 3 had significantly higher levels of SCD mRNA than those fed diet 1 ($p<0.001$, s.e.d. = 12.03). The subcutaneous depot had significantly more SCD mRNA than the other two depots for all diets ($p<0.001$, s.e.d. = 12.50) (Figure 1). In all depots there was a modest but highly significant increase in oleic acid content in the animals fed the concentrate diets and the subcutaneous depot had more oleate than the other depots for all diets. Although there was no difference in the amount of stearic acid between the diets in any depot there were significant differences between the depots with the perirenal having most and the subcutaneous the least (Table 1).

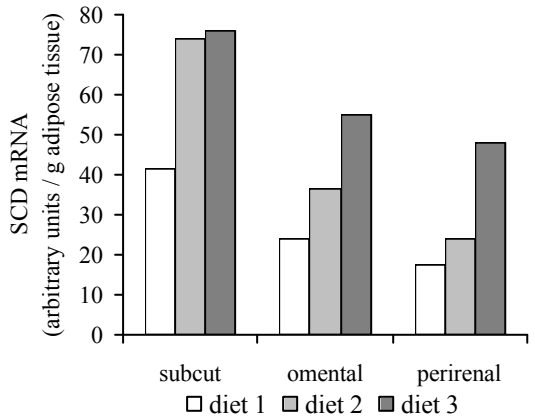


**Figure 1** Effect of diet and depot on SCD mRNA

**Table 1** Effect of diet and depot on fatty acid composition

| | | Fatty acid (mg/g adipose tissue) | | | | | |
|---|---|---|---|---|---|---|---|
| | diet | subcut | omental | perirenal | | s.e.d | sig. |
| stearate | 1 | 161.6 | 198 | 252.8 | diet | 14.64 | p=0.833 |
| | 2 | 165 | 200.9 | 229.7 | depot | 13.80 | p<0.001 |
| | 3 | 143.5 | 213.3 | 248.4 | | | |
| oleate | 1 | 214.4 | 183.7 | 181.7 | diet | 14.11 | p=0.006 |
| | 2 | 255.1 | 218.3 | 202 | depot | 12.22 | p<0.001 |
| | 3 | 243.9 | 217.5 | 210.8 | | | |

**Conclusion** These data show that feeding high levels of a barley-oat based diet to sheep increases SCD mRNA concentrations, perhaps by raising *in vivo* insulin levels. Although this increase in SCD expression was associated with a small increase in oleic acid content the proportion of stearic acid was unchanged.

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# NSP Enzymes For Broiler Diets Containing Barley

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**Introduction** Non-starch polysaccharide (NSP) content of barley is predominately in the form of β-glucan, whereas in wheat it is predominately pentosans (containing the sugars arabinose and xylose). It has generally been accepted therefore that a high level of β-glucanase is needed in broiler diets containing barley, whereas wheat based diets require a high level of endoxylanase. Trials have however previously suggested that including an endoxylanase based enzyme in broiler diets will perform at least as well as enzyme containing highlevels of β-glucanase in diets containing up to 30% barley. To confirm this finding, a trial was initiated at Roslin Institute, Edinburgh to compare the effectiveness of Natugrain Blend 66% (an endoxylanase based product) against Natugrain 33% (endoxylanase/β-glucanase product) in diets containing up to 30% barley.

**Materials & Methods** Four basal diets were prepared, with each containing a different level of barley (0%, 10%, 20% or 30%). All 4 diets were treated with recommended dosage levels of either Natugrain 33% L at 300g/t (to provide 825 endoxylase units, 600 βetaglucanases units per kg feed), Natugrain Blend 66% L at 150g/t (to provide 5,500 endoxylanase units, 120 βetaglucanase units per kg feed) or without enzyme addition. Each diet was replicated 8 times with a total of 40 birds per pen. The birds were fed a starter diet from 0-18 days and a corresponding finisher diet from 18-42 days of age. Diets were formulated to be isoenergetic (at 13.0 ME) and isonitrogenic (220g/kg crude protein), with barley specified at a poultry metabolizable energy value of 11.80 MJ/kg compared to 12.85 MJ/kg for wheat. Performance characteristics were subjected to analyses of variance and the standard errors of difference tested.

**Figure 1 Weight at 18 days (SEM[1] 8.4)**

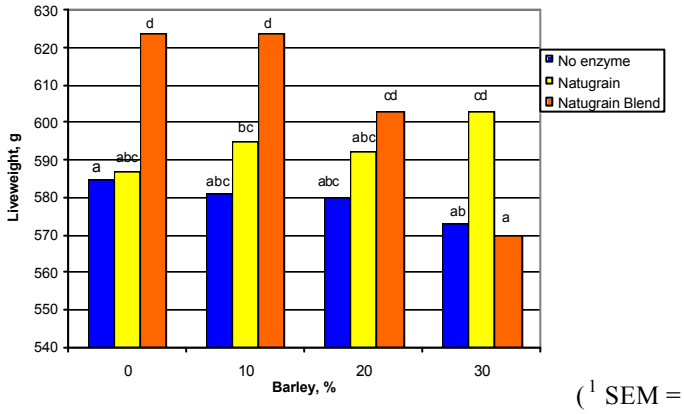
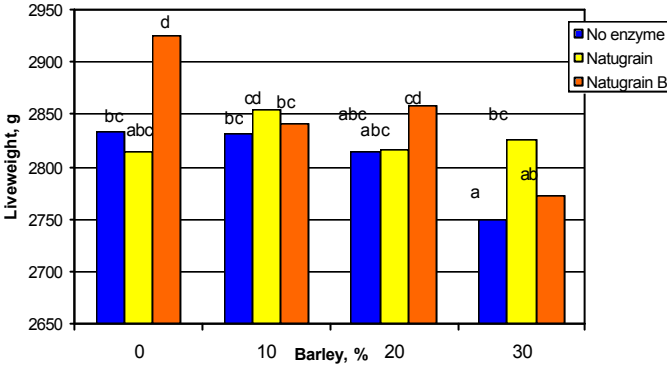
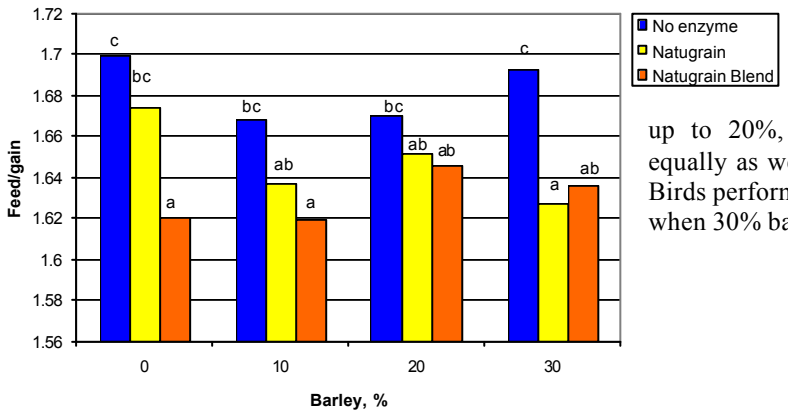



**Figure 2 Weight at 42 days (SEM[1] 26.6)**




([1] SEM = Pooled standard error of means)

**Figure 3 Feed Conversion 0-42 days (SEM[1] 0.014)**




**Results** Figures 1-3 show the performance of the birds throughout the trial. There were clear benefits shown when either enzyme was added to the diets. When barley was included up to 20%, birds fed Natugrain Blend 66% performed equally as well or better than the birds fed Natugrain 33%. Birds performed slightly better with the Natugrain 33% diets when 30% barley was included.

**Conclusion**

This data clearly shows that there are significant performance improvements when NSP-enzymes are included in diets containing wheat and barley.

The data shows that birds fed diets containing Natugrain Blend 66% (endoxylanase based product) and up to 20% barley performed better than Natugrain 33% (endoxylanase/β-glucanase product) in diets containing equivalent levels of barley.

There were clear performance advantages to the use of Natugrain Blend 66% in the all wheat diets compared to the control or Natugrain 33% treatments.

# Effects of incubation fluid pH and fibrolytic enzymes on the *in vitro* fermentation of pure substrates, assessed using the Reading Pressure Technique (RPT)

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**Introduction** Modern feeding practices often lead to ruminal conditions being sub-optimal for fibre digestion. It has been speculated that fibrolytic enzymes, which usually show optimum activity at pH values below 6.0, may be of benefit when applied to diets of high producing animals. This study used a commercial enzyme mixture (EM), already identified as effective; to investigate its optimum pH range with respect to activity and its impact on the fermentation profiles of pure substrates, under differing pH conditions.

**Methods** The EM used (*Liquicell 2500* (Specialty Enzymes and Biochemicals, CA, USA), had been extensively characterised (Colombatto *et al*., 2000). Determination of the optimum pH for xylanase and endoglucanase activity was carried out using birchwood xylan and carboxymethylcellulose as substrates, respectively. A 0.2 M citrate-phosphate buffer, adjusted to pH 4.0, 4.6, 5.0, 5.6, 6.2 and 6.8, was used and the activities were determined as described by Wood and Bhat (1988). The RPT system (Mauricio *et al*., 1999) was used for the *in vitro* fermentation study. Approximately 0.5 g of microcrystalline cellulose (Avicel PH-101, Fluka Chemicals), oat spelt xylan (Sigma Chemicals), and a mixture (AX, 50:50 w/w) of both was weighed in triplicate into fermentation flasks. The EM was applied at four levels, namely 0, 0.51, 2.55 and 5.1 μ/g substrate DM (0, 1x, 5x and 10x, respectively), 20 h prior to inoculation with rumen fluid. Four hours later, 90 ml of anaerobic buffer was added and the flasks stored at 20°C overnight. The buffer pH was adjusted 1 M citric acid solution, to provide initial pH values of 6.8, 6.2 and 5.8, respectively. Rumen fluid was collected pre-feeding (0700 h) from a dry cow fed grass hay. Controls containing rumen fluid only, and rumen fluid plus enzyme at the three levels were also included for correction. Gas readings were taken at 2, 4, 6, 8, 10, 12, 15, 19, 24, 30, 36, 48, 72 and 96 h post-inoculation. Rates and extent of gas production were determined. The study was completely randomised and data were analysed using the Mixed procedures of SAS.

**Results** The optimum pH for xylanase activity was found to be in the 5.2-5.6 range, whereas a strong inverse relationship was found for endoglucanase activity (optimum pH was 4.0). Irrespective of pH, addition of EM significantly increased ($P<0.01$) the rate and extent of gas production in all substrates studied (Table 1). After 48 h incubation, the proportional improvement in gas production due to EM addition was much more marked at lower pH values, when averaged across enzyme levels. The latter supports the idea that fibrolytic enzymes may have a greater impact when ruminal conditions are sub-optimal for fibre digestion.

**Table 1**. *Cumulative gas production (ml/g OM) of enzyme-treated Avicel, Xylan and a mixture of both (AX)*

| | | pH 5.8 | | | pH 6.2 | | | pH 6.8 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Substrate | EM level | 24 h | 48 h | 96 h | 24 h | 48 h | 96 h | 24 h | 48 h | 96 h |
| Avicel | 0 | 16.5$^{ab}$ | 17.7$^{ab}$ | 71.5$^{ab}$ | 15.8$^{a}$ | 119.0$^{a}$ | 203.7$^{a}$ | 102.1$^{a}$ | 259.1$^{a}$ | 317.3$^{a}$ |
| | 1x | 5.3$^{a}$ | 10.8$^{a}$ | 69.8$^{a}$ | 31.3$^{b}$ | 157.9$^{c}$ | 252.7$^{b}$ | 113.7$^{a}$ | 295.6$^{bc}$ | 354.9$^{b}$ |
| | 5x | 25.7$^{b}$ | 32.3$^{ab}$ | 97.8$^{b}$ | 19.4$^{a}$ | 144.9$^{b}$ | 236.4$^{b}$ | 132.8$^{b}$ | 309.8$^{c}$ | 362.9$^{b}$ |
| | 10x | 28.7$^{b}$ | 36.1$^{b}$ | 95.8$^{ab}$ | 18.7$^{a}$ | 139.7$^{ab}$ | 235.8$^{b}$ | 104.1$^{a}$ | 284.7$^{b}$ | 343.3$^{ab}$ |
| s.e.d. | | 7.34 | 10.89 | 13.49 | 7.34 | 10.89 | 13.49 | 7.34 | 10.89 | 13.49 |
| Xylan | 0 | 110.1$^{a}$ | 129.6$^{a}$ | 147.5$^{a}$ | 157.6$^{a}$ | 196.3$^{a}$ | 233.1$^{a}$ | 162.9$^{a}$ | 248.4$^{ab}$ | 287.7$^{a}$ |
| | 1x | 129.8$^{ab}$ | 147.1$^{b}$ | 158.6$^{ab}$ | 180.1$^{b}$ | 223.4$^{c}$ | 267.2$^{c}$ | 171.1$^{ab}$ | 265.1$^{bc}$ | 306.3$^{b}$ |
| | 5x | 131.3$^{b}$ | 161.7$^{bc}$ | 179.5$^{c}$ | 179.9$^{b}$ | 221.1$^{bc}$ | 258.1$^{bc}$ | 188.4$^{b}$ | 277.4$^{c}$ | 314.7$^{b}$ |
| | 10x | 162.6$^{c}$ | 166.8$^{c}$ | 174.1$^{bc}$ | 171.4$^{ab}$ | 204.6$^{ab}$ | 247.1$^{ab}$ | 158.6$^{a}$ | 245.7$^{a}$ | 279.7$^{a}$ |
| s.e.d. | | 10.14 | 8.32 | 8.58 | 10.14 | 8.32 | 8.58 | 10.14 | 8.32 | 8.58 |
| AX | 0 | 60.7$^{a}$ | 69.8$^{a}$ | 106.6$^{a}$ | 76.1$^{a}$ | 155.6$^{a}$ | 229.6$^{a}$ | 126.7$^{a}$ | 272.9$^{a}$ | 316.6$^{a}$ |
| | 1x | 83.3$^{b}$ | 97.2$^{b}$ | 128.1$^{bc}$ | 92.9$^{b}$ | 178.9$^{b}$ | 255.2$^{bc}$ | 132.7$^{a}$ | 285.5$^{a}$ | 328.6$^{a}$ |
| | 5x | 52.1$^{a}$ | 87.9$^{b}$ | 123.4$^{b}$ | 99.6$^{b}$ | 188.5$^{b}$ | 266.2$^{c}$ | 153.1$^{b}$ | 306.8$^{b}$ | 349.3$^{b}$ |
| | 10x | 114.5$^{c}$ | 118.2$^{c}$ | 141.2$^{c}$ | 93.3$^{b}$ | 177.6$^{b}$ | 249.8$^{b}$ | 124.5$^{a}$ | 273.7$^{a}$ | 313.8$^{a}$ |
| s.e.d. | | 7.04 | 7.46 | 8.06 | 7.04 | 7.46 | 8.06 | 7.04 | 7.46 | 8.06 |

Within columns and substrates, means without common superscripts differ significantly ($P<0.05$).

**Conclusions** Addition of a fibrolytic enzyme mixture increased the rate and extent of fermentation of pure substrates *in vitro*. The degree of improvement was inversely related to the pH of the incubation fluid, and increased with low to medium EM levels. This may have implications for the study of the mode of action of enzymes as feed additives for ruminants.

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# The effect of fibrolytic enzyme application on the rate and extent of alfalfa stem fermentation, assessed *in vitro*

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**Introduction** A number of fibrolytic enzyme preparations have been shown to increase the rate and extent of fermentation of alfalfa fractions (Colombatto *et al*., 2000a). However, responses to enzyme addition have been mixed and several factors are believed to be involved. Among these, specific enzyme activities and application rates are very important. The present study examined a commercial enzyme preparation already established as effective, for its ability to increase the rate and extent of *in vitro* fermentation of alfalfa stems, when applied at different levels.

**Methods** The enzyme preparation (*Liquicell 2500* (Specialty Enzyme and Biochemicals, CA, USA) was fully characterized prior to use (Colombatto *et al*., 2000b). Per ml of enzyme preparation, and determined at 39°C and pH 5.5, contained 14864, 1699, 2.6 and 45.5 units of xylanase, endoglucanase, exoglucanase and β-glucosidase activity, respectively. The Reading Pressure Technique (RPT, Mauricio *et al*., 1999) was used for the study. Approximately 1 g DM of alfalfa stems, pre-dried and milled to pass a 2 mm screen, was weighed in triplicate into 125-ml capacity fermentation flasks. *Liquicell 2500* was then added at six levels, namely 0 (L0), 0.51 (L1), 1.02 (L2), 2.55 (L3), 5.10 (L4), and 25.50 (L5) µl/g forage DM. Three hours later, 90 ml anaerobic buffered was added to the flasks, and they were stored at 20-22°C for 17 h. Ten ml prepared rumen fluid, obtained pre-feeding (0700 h) from a dry cow fed on grass silage, was inoculated into the flasks. Negative controls containing buffered rumen fluid with or without enzyme but no substrate, were also included in triplicate for correction. Head-space gas readings (GP) were taken at 2, 4, 6, 8, 10, 12, 15, 19, 24, 30, 36, 48, 72 and 96 h post incubation at 39°C. Complete sets of treatments were withdrawn at 6, 12, 19, 24, 48 and 96 h, filtered and dried (100°C for 24 h) to determine DMD and OMD (following ashing at 500°C for 6 h). A completely randomized design was used, and data were analyzed using Mixed Procedures of SAS.

**Results** Enzyme addition linearly increased (P<0.01) cumulative GP up to 12 h post-inoculation. No differences (P>0.05) were observed thereafter, suggesting that enzymes increased the initial rate, but not extent, of alfalfa fermentation. Consistently, linear increases (P<0.01) in OMD up to 19 h were observed with increasing levels of enzyme addition (Table 1). However, no differences (P>0.05) were found on the end-point (96 h) OMD.

**Table 1.** *Effect of enzyme addition on cumulative GP (ml/g OM) and Organic matter degradation (OMD, g/kg)*

| | Cumulative gas | | | | OMD | | | |
|---|---|---|---|---|---|---|---|---|
| Treatment | 12 h | 19 h | 24 h | 96 h | 12 h | 19 h | 24 h | 96 h |
| L0 | 68.9$^{a}$ | 116.8$^{a}$ | 136.3$^{a}$ | 179.4$^{ab}$ | 348.7$^{ab}$ | 425.3$^{a}$ | 469.6$^{a}$ | 552.4$^{a}$ |
| L1 | 70.9$^{ab}$ | 120.8$^{a}$ | 141.6$^{a}$ | 187.6$^{a}$ | 347.2$^{a}$ | 443.8$^{bc}$ | 476.2$^{ab}$ | 559.6$^{a}$ |
| L2 | 67.6$^{a}$ | 116.7$^{a}$ | 137.2$^{a}$ | 179.0$^{ab}$ | 353.0$^{abc}$ | 433.5$^{ab}$ | 476.7$^{ab}$ | 557.3$^{a}$ |
| L3 | 67.9$^{a}$ | 115.3$^{a}$ | 135.6$^{a}$ | 174.8$^{b}$ | 341.7$^{a}$ | 445.6$^{c}$ | 482.8$^{b}$ | 557.1$^{a}$ |
| L4 | 71.1$^{ab}$ | 117.3$^{a}$ | 136.2$^{a}$ | 176.9$^{b}$ | 360.9$^{bc}$ | 442.9$^{bc}$ | 466.0$^{a}$ | 555.5$^{a}$ |
| L5 | 74.9$^{b}$ | 120.6$^{a}$ | 138.9$^{a}$ | 182.1$^{ab}$ | 365.5$^{c}$ | 450.6$^{c}$ | 474.9$^{ab}$ | 559.4$^{a}$ |
| s.e.d. | 2.35 | 2.66 | 3.07 | 4.41 | 6.30 | 4.94 | 5.80 | 5.68 |
| Linear | 0.008 | 0.181 | 0.659 | 0.690 | 0.005 | 0.003 | 0.968 | 0.517 |
| Quadratic | 0.965 | 0.365 | 0.294 | 0.085 | 0.296 | 0.038 | 0.402 | 0.939 |

Means within columns without common superscripts differ (P<0.05). [§] Probability of significant effects

**Conclusions** Addition of a commercial enzyme preparation increased the *in vitro* initial rate of gas production and degradability of alfalfa stems. Moreover, the effects were linearly related to increasing levels of enzyme during the first 19 h incubation. The latter suggests that enzymes increased the hydrolytic potential of rumen fluid. The RPT technique is capable of identifying slight changes in fermentation activity due to enzyme additions.

**Acknowledgements** FOMEC Program (Facultad de Agronomía, University of Buenos Aires, Argentina) and BBSRC.

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# Screening of fibrolytic enzymes as additives for ruminant diets: relationship between enzyme activities and the *in vitro* degradation of enzyme-treated forages

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**Introduction** Results in the literature concerning the efficacy of feed enzymes for ruminant diets have been mixed. Commercial preparations currently used are fermentation extracts containing several enzymic activities. It has been suggested that ruminal fermentation of grass and maize silages is enzyme-limited (Wallace *et al*., 2001). In order to design better enzyme additives, the enzyme activities likely to affect the animal responses should be identified. This study examined 23 commercial enzyme preparations for their biochemical properties and their ability to influence the *in vitro* degradation of alfalfa and maize silage.

**Methods** Twenty three commercial enzyme mixtures (EM) were used in this study. Protein contents were determined using the BioRad DC Protein assay. Activities against oat spelt xylan, birchwood xylan, carboxymethylcellulose (CMC), microcrystalline cellulose, starch, β-glucan, xyloglucan, arabinogalactan, lichenan, arabinoxylan, laminarin, *p*-nitrophenyl (*p*-NP) acetate, *p*-NP-α-L-arabinofuranoside, *p*-NP-β-D-glucopyranoside, *p*-NP-β-D-xylopyranoside and *p*-NP-β-D-galactoside were determined at pH 6.0 and 39°C. β-glucan, xyloglucan, arabinogalactan and arabinoxylan were obtained from Megazyme Int. (Ireland), whereas all other substrates were obtained from Sigma Chemicals (St. Louis, MO, USA). Assays were conducted in triplicate, and blanks containing substrate only or enzyme only were included in triplicate for correction. Specific activities were determined for each preparation by relating their activities to protein concentration. The hydrolytic potential of the preparations was determined in triplicate by measuring the reducing sugars released from alfalfa hay or maize silage after 15 min incubation at 39°C and pH 6.0 with enzyme preparation. Substrates had been washed with distilled water for 2 h to extract the soluble carbohydrates prior to use. Again, blanks were included for correction. For the *in vitro* rumen degradation study, approximately 1 g untreated alfalfa hay or maize silage was weighed in triplicate into fermentation flasks. Enzymes (1.5 mg/g DM forage) were added to the flasks 20 h before inoculation with rumen fluid. Three hours later, 40 ml anaerobic buffer (adjusted to pH 6.0 using 1 M trans-aconitic acid) was added, and the flasks were stored at 25°C overnight. Rumen fluid was obtained pre-feeding from three lactating cows fed a maize silage-based diet, and 10 ml was inoculated into each flask. Incubation proceeded for 18 h, and undegraded residues were filtered into crucibles, dried at 109°C for 24 h to determine dry matter degradation (DMD). Protein contents, each total and specific enzyme activity, and reducing sugars released were correlated to the DMD values for each substrate using the Stepwise Regression Procedures of SAS. Analyses were conducted separately for total and specific activities.

**Results** The EM analyzed varied widely in their protein contents, activity arrays and hydrolytic capacity. Comparisons of the effects on degradation of both substrates with protein contents, total activities and reducing sugars released indicated that the strongest correlation ($P<0.05$) was with activity against xylan from oat spelts (Table 1). No correlation was found between the hydrolytic potential of the enzymes and their performance in presence of rumen fluid. However, the relationship was positive with alfalfa but negative with maize silage. When the correlations were performed using specific activities alfalfa DMD was negatively correlated with specific activity against xyloglucans, whereas maize silage was positively correlated with activity against CMC and negatively to *p*-NP-β-D-glucopyranoside.

**Table 1**. Relationship between total and specific activities and the *in vitro* DMD of alfalfa hay or corn silage

| Parameter | Forage | Substrate | Relationship | Partial $R^2$ | Model $R^2$ | $P > F$ |
|---|---|---|---|---|---|---|
| Total activities | Alfalfa hay | Oat spelt xylan | y = 0.042 x + 453.6 | 0.29 | 0.29 | 0.010 |
| | Maize silage | Oat spelt xylan | y = -0.033 x + 446.6 | 0.19 | 0.19 | 0.044 |
| Specific activities | Alfalfa hay | Xyloglucan | y = -28.385 x + 464.9 | 0.20 | 0.20 | 0.032 |
| | Maize silage | CMC | y = 18.896 x + 436.0 | 0.28 | | 0.008 |
| | | p-NP-β-D-glucopyranoside | y = -80.541 x + 437.3 | 0.08 | 0.36 | 0.128 |

[§] Model substrate used to calculate enzyme activity

**Conclusions** Results from this study suggest that there is some relationship between the enzyme activities determined on model substrates and the nature of the responses when these enzymes are used as additives in ruminant diets. However, the nature of the relationship varied depending upon the forage, and whether total or specific activities were used in the calculations. Further research with more enzyme preparations, and possibly purified enzymes, is warranted to confirm these results.

**Acknowledgements** Cargill Corp. is acknowledged for providing the enzyme preparations.

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# Effect of soluble non starch polysacharide degrading enzyme supplements on nutrient efficiency of young broiler chickens fed wheat with different viscosities and triticle

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**Introduction** Although wheat is an important ingredient in poultry diets, a large variability of the nutritive quality of wheat especially in its AME value is reported. A negative correlation between the lower AME in wheat or other cereal grains like barley, rye and triticle and their amount of soluble non-starch polysacharides (NSP) has been found in birds (Annison, 1990). A positive correlation between the amount of NSP in grains and the viscosity of the gut is also reported (Van der klis *et al*., 1995). High viscosity of the gut reduces the performance of the birds. Detrimental effect of NSP can be decreased by adding NSP degrading enzymes in the diet (Annison, 1992). Therefore the objective of this experiment was to study the viscosity of different wheat cultivars and triticle and the effect of NSP degrading enzyme supplements in young broiler chickens.

**Materials and Methods** To *an in vitro* experiment, different varieties of wheat were tested and the highest (flaat) and lowest (ghods) viscosities were selected. 288 day-old Arian broiler chickens were kept on cages and four grains (two wheat varieties, Flaat, and Ghods; triticle and corn) with (+) or without (-) a dietary NSP degrading enzyme supplement (Endofeed W) were added to a basal diet with 60% of each grain and fed to broiler chickens from 1-21 days of age. Feed and water provided *ad libitum.* To measure the nutrient digestibility of the experimental diets, an indigestible marker (chromic oxide) was used and feces samples were collected from 18-21 days of the experiment. Experimental design was CRD with a 4*2 factorial arrangement with 6 replicates per treatment. Data were analyzed using the general linear model procedure of SAS (1986).

**Results** Apparent metabolizable energy corrected for nitrogen ($AME_n$), apparent lipid digestibility (ALD), apparent crude carbohydrate digestibility (ACCD) and nitrogen retention (NR) are shown in table. AMEn in all treatments except in corn diet affected by enzyme supplement (P<.01). ALD and ACCD of all treatments were significantly improved by adding enzyme (P<.01). This improvement in Flaat with the highest viscosity was highest. NR was also improved when enzyme added to each grain (P<.01).

**Table** Effects of dietary treatments on AMEn (kcal/kg) and nutrient digestibilities (%) in broiler chickens from 18-21 days of age (dry matter basis)

| | | Experimental diets with 60% of each grain | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Corn | | Triticle | | Ghods | | Flaat | | |
| P values | ±SEM | + | - | + | - | + | - | + | - | |
| <.01 | 31.3 | $3276^{ef}$ | $3211^{f}$ | $3455^{bc}$ | $3344^{de}$ | $3500^{b}$ | $3393^{cd}$ | $3664^{cde}$ | $3354^{a}$ | AMEn |
| <.01 | 0.63 | $80.1^{a}$ | $76.6^{bc}$ | $76.0^{c}$ | $69.6^{d}$ | $74.6^{c}$ | $70.9^{d}$ | $78.2^{ab}$ | $65.5^{e}$ | ALD |
| <.01 | 0.21 | $84.7^{a}$ | $83.6^{b}$ | $81.1^{d}$ | $79.5^{f}$ | $83.5^{b}$ | $82.1^{c}$ | $83.3^{b}$ | $80.4^{e}$ | ACCD |
| NS | 0.38 | 68.3 | 65.4 | 66.6 | 61.4 | 64.7 | 61.3 | 70.2 | 66.4 | NR |

P values for enzyme and diet effects were significantly different (P<.01). AMEn, apparent metabolizable energy corrected for nitrogen; ALD, apparent lipid digestibility; ACCD, apparent crude carbohydrate digestibility; NR, nitrogen retention. +, with enzyme; -, without enzyme. The values in each row with different superscripts are significantly different (P<.01).

**Conclusions** Under the conditions of this experiment, it was concluded that addition of NSP degrading enzyme to diets containing wheat and triticle increases their AMEn, ACCD and especially ALD. The highest improvement can be achieved for those with the highest *in vitro* viscosity (Flaat and triticle). Therefore, the extract viscosity of the wheat and triticle would be a suitable index for predicting the nutritive value of such grains.

**Acknowledgement** Financial support of Ferdowsi University of Mashhad, Iran is greatly acknowledged.

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# Yeast application and its derived products to reduce aflatoxicosis

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**Introduction**, Yeasts have been studied and used as source of proteins for animal feeding over many years. Recently, new ideas on their use has lead to studies to enrich yeasts with micronutrients and use them as vehicles in feed production. Yeast cellular components are also used to improve the animal health and performance. The use of living yeasts are also thought to improve animal food consumption and reduce cell damage caused by substances such as aflatoxins found in animal foods. Aflatoxins, secondary metabolites produced by some fungi in foods and feeds can, on ingestion, result in the illness or death of animals. The aim of this study was to investigate the effects of *Saccharomyces cerevisiae* living cells and its derived products on animals when they are supplied with diets contaminate by aflatoxins.

**Materials and Methods A**an experiment was carried out using a completely randomised design, with six treatments and five replications. It used 30 "Wistar" rats aging 21-25 d and 45 – 50 g LW. Six formulations were obtained based on a basal diet, AIN-93, described by Reeves et al. (1993). The treatments evaluate were: T1 (basal diet), T2 (basal diet + 400 μg $kg^{-1}$ aflatoxins), T3 (basal diet + 400 μg $kg^{-1}$ aflatoxins + 1% of yeast termolised), T4 (basal diet + 400 μg $kg^{-1}$ aflatoxins + 0,1% of mananoligossacarides), T5 (basal diet + 400 μg $kg^{-1}$ aflatoxins + 0,2% of mananoligossacarides) and T6 (basal diet + 400 μg $kg^{-1}$ of aflatoxins+ 1% of living yeasts). Peanuts with aflatoxins were used to contaminate the diets. Aflatoxins analysis was carried out using a thin layer chromatography technique (Soares & Rodrigues-Amaya, 1989). The experiment took 28 days, and each animal was supplied with water *ad libitum* and 15 grams of diet depending on treatment. The parameters investigated were: internal organs relative weight (heart, kidneys and liver) and fragments of the hepatic tissue. In the histological analysis, animal hepatic tissues submitted to T1 were adopted as a reference due to being free of toxicity. The internal organs relative weight was submitted to an analysis of variance and the averages were compared by Tukey test.

**Results** The internal organs relative weight submitted to different treatments did not differ statistically (Table 1). Animals submitted to diet T2 showed in their livers evident of toxicity ("vacuolisation" and proliferation of epithelial cells of the bile duct). Animals that received diets T3 and T4 also showed sign of toxicity, similar to animals fed with T2 diet. Animals submitted to diet T5 showed higher levels of toxicity than animals fed with diet T2; a large area of liver tissue from group T5 was observed with vacuolisation, proliferation of epithelial cells of the bile duct and hyalinisation. However, animals that received T6 diet showed signs of reduced toxicity, with little vacuolisation and little proliferation of epithelial cells of the bile duct. The last treatment showed evident of low toxicity with similar results to the T1 group.

**Table 1**– Internal organs relative weight of Wistar rats fed with different treatments

| Treatments | Liver | Heart | Kidneys |
|---|---|---|---|
| | Relative weight g/100g | | |
| T1 | 3.26 | 0.46 | 0.94 |
| T2 | 3.46 | 0.41 | 0.79 |
| T3 | 3.66 | 0.44 | 0.78 |
| T4 | 3.39 | 0.42 | 0.87 |
| T5 | 3.42 | 0.49 | 0.82 |
| T6 | 3.63 | 0.44 | 0.77 |
| SE | 0.39 | 0.07 | 0.10 |

**Conclusions** The results of this study demonstrated that treatments with termolised yeasts and mananoligossacarides did not decrease cell damage caused by aflatoxins**.** Living yeasts were however able to reduce toxicity damage caused by aflatoxins. Diets need to contain 1% living yeasts to have a noticeable reduction in cell damage caused by aflatoxins.

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# Influence of selected fibrolytic enzymes on the ensiling characteristics and *in vitro* rumen degradation of maize silage

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**Introduction** Selected fibrolytic enzyme preparations applied at ensiling have been shown to reduce the fibre contents and to increase the initial rate of *in vitro* organic matter degradation (OMD) of maize silage (Colombatto *et al*., 2001). However, there is little information on changes in the fibre content of maize forage during the ensiling process, as affected by enzyme addition. The present study examined the effects of characterised enzyme preparations (Colombatto *et al*., 2000), derived from mesophilic and thermophilic fungal sources applied at ensiling, on the quality and *in vitro* rumen degradation characteristics of maize silage, as assessed using the Reading Pressure Technique (RPT, Mauricio *et al*., 1999).

**Methods** Forage maize (333 g/kg DM) containing 70, 470, 227, 243, 307 and 47 g/kg DM protein, NDF, ADF, hemicellulose, starch and WSC, respectively, was ensiled in 0.5 kg minisilos. Prior to ensiling, the forage was left untreated or treated with the mesophilic preparation *Liquicell 2500* (L, Specialty Enzymes and Biochemicals, CA, USA), derived from *Trichoderma reesei*, or a crude thermophilic extract derived from *Thermoascus aurantiacus* (Ta, produced by the authors). Application levels (1 and 2) provided 11500 and 23700 units xylanase activity/kg forage DM, respectively (determined as described in Colombatto *et al*., 2000). Silos were stored at 15-20°C, and duplicate silos were opened 2, 4, 8, 15, and 60 d post ensiling. Silage pH, NDF, ADF, hemicellulose, starch and WSC contents were determined. Composite samples from the 60 d silages were used in triplicate for the *in vitro* gas production (GP) and OMD study. Approximately 1 g DM was added to each fermentation flask. Rumen fluid was collected pre-feeding (0700 h) from a dry cow fed on a maize silage-based TMR. Gas readings were taken at 2, 4, 6, 8, 10, 12, 15, 19, 24, 30, 36, 48, 72 and 96 h post incubation, and complete sets of treatments were removed after 6, 12, 19, 24, 48 and 96 h for OMD determination. Negative controls containing rumen fluid and anaerobic buffer alone were also included in triplicate at each removal time. A 2 x 2 factorial arrangement was used, with enzyme and level as main factors. The data were analysed using GLM procedures of SAS.

**Results** Enzyme-treated silages showed significantly lower ($P<0.05$) pH values, with the differences ($P<0.05$) observed from the early stages of the fermentation (Table 1). The pH continued to drop after 15 d ensiling, suggesting that the enzymes were still active during this period. NDF and ADF contents were reduced ($P<0.05$) after 4 d with L 2. ADF contents were reduced ($P<0.05$) in all treated silages at 60 d. As a result, starch contents proportionally increased in the enzyme-treated silages. Initial GP rates were increased ($P<0.05$) in L 1 and the two Ta treatments. Consistently, 24 h OMD values were increased ($P<0.05$) in all treated silages. Final (96 h) was only increased ($P<0.05$) in Ta 1, however all other treated silages showed numerical differences compared to the controls (Figure 1).

**Table 1.** Effects of enzyme addition on pH and NDF, ADF and Hemicellulose (HC) contents (g/kg DM) of maize silage

| | 4 days | | | | 60 days | | | |
|---|---|---|---|---|---|---|---|---|
| Treatment | pH | NDF | ADF | HC | pH | NDF | ADF | HC |
| Control | 3.91$^{a}$ | 486$^{a}$ | 245$^{a}$ | 241$^{a}$ | 3.94$^{a}$ | 470$^{a}$ | 232$^{a}$ | 238$^{a}$ |
| L 1 | 3.83$^{b}$ | 423$^{ab}$ | 202$^{ab}$ | 221$^{ab}$ | 3.69$^{b}$ | 415$^{a}$ | 200$^{c}$ | 214$^{a}$ |
| L 2 | 3.84$^{b}$ | 403$^{b}$ | 195$^{b}$ | 207$^{b}$ | 3.77$^{b}$ | 458$^{a}$ | 216$^{b}$ | 241$^{a}$ |
| Ta 1 | 3.83$^{b}$ | 451$^{ab}$ | 224$^{ab}$ | 227$^{ab}$ | 3.72$^{b}$ | 451$^{a}$ | 209$^{bc}$ | 242$^{a}$ |
| Ta 2 | 3.83$^{b}$ | 448$^{ab}$ | 226$^{ab}$ | 222$^{ab}$ | 3.70$^{b}$ | 432$^{a}$ | 207$^{bc}$ | 225$^{a}$ |
| s.e.m. | 0.005 | 20.3 | 13.3 | 7.6 | 0.027 | 19.2 | 3.9 | 17.1 |

Means with unlike superscripts differ ($P<0.05$)

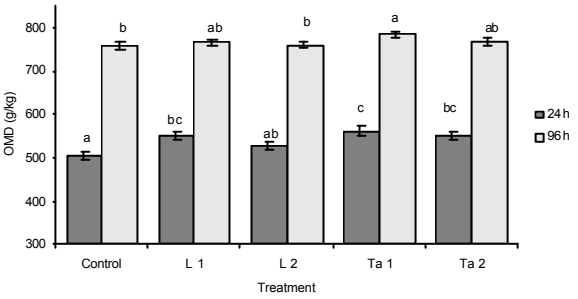


**Figure 1.** Initial (24 h) and final (96 h) *in vitro* OMD of enzyme-treated maize silages

**Conclusions** Addition of selected enzyme preparations prior to ensiling reduced the fibre contents in maize and increased the initial *in vitro* OMD. The implication of this finding needs to be confirmed *in vivo*.

**Acknowledgements** Financial support from University of Buenos Aires (FOMEC Program) and BBSRC.

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# The effect of castration on plasma cortisol level and time budget in farmed guanaco calves (*Lama guanicoe*) ♣

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**Introduction** Castration is a routine procedure for male farm animals. The ethics of castration are widely debated because the procedure may potentially result in pain and distress. The indications for early castration in farmed guanacos are: 1) prevention of aberrant behaviour in human-imprinted males, 2) elimination of inter-male aggression, so that males may be housed together or with females, 3) avoidance of accidental breeding (Fowler, 1998). In addition, it has been argued (Molony and Kent, 1997) that to study the pain response to castration is ethically acceptable as the overall welfare of the animal is improved by the procedure. Guanaco farming has been recently established in South America, and there is therefore little information available on how guanacos respond to castration. The purpose of this study was to assess pain in farmed guanacos. The hypothesis was that pain experienced by guanacos due to castration may be displayed in behavioural and physiological changes, as has been reported in lambs, calves and piglets. The study was carried out in 2001 on four-month-old farmed male guanaco. Changes in plasma cortisol concentration and the frequency of specific daytime behavioural postures and activities following castration are reported.

**Materials and Methods** Nine male guanacos (four months old, 31.8 ± 7.0 kg live weight) were used in this study. The animals were randomly allocated to one of two treatment groups: 1) handling (control) (n= 4) and 2) handling + castration (n=5).The animals were castrated using a surgical procedure (see Fowler, 1998). Both groups were infiltrated with local anaesthetic (3 ml lidocaine 2%). Plasma cortisol concentration (nmol/L) and the frequency (%) of five behavioural categories: walking (W), suckling (S), foraging (F), standing (St), lying (L), were monitored. Blood samples were taken from the jugular vein one week before castration, immediately before castration, then 2 hours, 24 hours and one week after castration. The average of the first and second sampling before castration was used as the baseline level. The frequency of behaviour was recorded between 11:00 and 17:00 for 3 days prior to castration, and for 5 days after castration. Plasma cortisol was analysed by ANOVA with repeated measurements (treatment as a factor, and simple contrast with the first level as baseline). MANOVA of the frequency of behavioural categories was conducted by day of study. P values of <0.05 were considered significant.

**Results** A significant effect of castration on plasma cortisol level was recorded (Table 1). Cortisol concentration in castrated guanacos peaked 4 hours after castration at 1.8 times the baseline level. It remained high for up to 24 hours, and returned to baseline one week after castration. In control guanacos, except for a fall 4 hours post handling, there were no significant differences in cortisol concentration compared to baseline level. Castrated animals spent more time lying down on the day of the operation, compared with the control (Table 1). No other behavioural changes were observed.

Table 1. Mean ± S.E.M. of cortisol and time budget in control and castrated guanacos

| | Mean ± S.E.M. Cortisol (nmol/L) | | | | | Frequency of behavioural categories ± S.E.M. (%) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Baseline | 4 h | 24 h | 1 week | Total | Walking | Standing | Suckling | Foraging | Lying |
| Control (n= 4) | 20.7 ±6.90 | 7.0 ±1.44a | 27.9 ±10.02 | 16.3 ±4.82 | 16.8 ±4.51 | 12.9 ±2.95 | 15.3 ±6.45 | 10.5 ±2.22 | 38.8 ±5.70 | 17.3 ± 6.10* |
| Castrated (n= 5) | 28.3 ±6.70 | 51.1 ±8.07a | 49.6 ±8.42a | 32.2 ±3.72 | 47.0 ±6.09* | 12.6 ±3.37 | 8.8 ±2.13 | 7.6 ±3.33 | 25.8 ±8.1 | 45.2± 8.70 |

a= significant difference from baseline level, p<0.05; * significant difference between treatment groups, p<0.05).

**Conclusions** Pain following surgical castration produces an increase in plasma cortisol concentration and time spent lying down in farmed male guanaco calves. The observed changes were transient, indicating that the pain experienced is temporary. It is concluded that the use of analgesics should be considered for post operative care in castrated guanacos. Further studies are needed to confirm this.

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♣ This study was funded by The Wellcome Trust 057689/Z/99/Z and the Chilean Ministry of Agriculture (FIA, project N° c98-1-p-019

# Domestic chicks' responses to PECKA-BLOCKS and string enrichment devices

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**Introduction** The PECKA-BLOCK (Breckland International Ltd.) is a commercially available, cereal and baked-sand-based device designed to enrich the environment and reduce the incidence of behavioural vices, such as feather pecking and cannibalism. Pecking at this novel stimulus loosens cereal grains that can then be eaten. This device reduced inter-bird pecking in broiler chicks (Guy, 2001) though it is not clear whether the authors measured gentle or severe feather pecking, allopreening, or aggression. White string (polypropylene twine) elicits sustained interest by chicks and hens of various laying and its provision reduced feather pecking and pecking-related feather damage (Jones, 2001). The present study compared the responses of layer chicks to simple string devices and to soft and hard PECKA-BLOCKS.

**Materials and Methods** Thirty-eight female Lohmann Brown chicks were housed in pairs in wooden boxes at ≅ 28 °C with a 14-hour photoperiod and ad libitum access to food (starter mash) and water. At 4 days of age they were acclimatised to the presence of an overhead micro-camera. One randomly selected chick in each pair was marked on the head to aid its identification as the focal chick. At 5 days, three 8 x 3.5 cm devices were suspended simultaneously from the tops of 3 walls: a standard, hard Peckablock (PB), a soft PB that facilitated loosening of cereal and that was produced specially for this trial, and a bunch of string (white polypropylene twine); these remained in place for 7 days. The birds' responses were recorded onto videotape using an overhead microcamera for 5 min in the morning and afternoon of each day. Upon subsequent analysis of the videotapes we counted the numbers of pecks directed at each of the stimuli. These scores were summed across the 14 observation periods and then averaged to yield a single measure for each chick. The Friedman two-way analysis of variance was used to determine if the chicks responded differently to the 3 pecking stimuli. The pattern of interest in these devices was examined by comparing pecking at each one on the first and seventh day of presentation using the Wilcoxon signed ranks test.

**Results** The chicks pecked readily at the hard PB, the soft PB and the string devices (overall means ± standard errors of 0.80 + 0.20, 1.15 + 0.19, 3.30 + 2.80, respectively). No significant differences were found. All three stimuli were equally attractive on initial presentation but interest had decreased by day 7 in each case though this decline was significant only for the hard PB.

**Table 1** Numbers (no) of pecks at the devices on Days 1 and 7 (means + standard errors)

| Device | Day 1 | Day 7 | P |
|---|---|---|---|
| Hard PB (no) | 1.40 + 0.61 | 0.08 + 0.06 | <0.02 |
| Soft PB (no) | 1.37 + 0.64 | 0.74 + 0.31 | =0.50 |
| String (no) | 1.29 + 0.71 | 0.53 + 0.16 | =0.65 |

**Conclusions** String and PECKA-BLOCKS elicited similar amounts of pecking on the first day of presentation. The chicks' interest in the devices decreased after 7 days continuous exposure, presumably via habituation, though the rates of decline differed. Chicks often tease apart the strands of string devices as if they were preening them (Jones, 2001) and the crumbly texture of the soft PB allowed them to break off and eat pieces of cereal. Therefore, these stimuli may have provided positive feedback to counter the effects of habituation. Conversely, pecking at the hard PB may have been less rewarding because of the difficulty in breaking off cereal; this may have compounded habituation. String devices may represent an alternative / additional and inexpensive source of environmental enrichment.

**Acknowledgements** This study was supported by DEFRA. We are also grateful to R.A. Phillips (Breckland International Ltd) for supplying the PECKA-BLOCKS.

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# The presence of a familiar odourant increases social affiliation when pairs of unfamiliar chicks are tested in a novel environment

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**Introduction** Chickens can differentiate between cagemates and strangers and they are often exposed to unfamiliar birds in novel environments under modern farming practice; this can lead to xenophobia, aggression and distress (Rajecki et al., 1976; Jones, 1996). Chickens form olfactory memories and regulate their behaviour in response to naturally occurring and artificial odours (Jones & Roper, 1997). The presence of a familiar odourant (vanillin) increased social dispersal and feeding when familiar pairs of chicks from the same cage were tested in a novel environment (Jones et al., 2001); suggesting that familiar odourants can be reassuring. Here, we asked if the presence of a familiar odour (vanillin) would increase social affiliation when two unfamiliar pairs of chicks were placed in a novel test arena.

**Methods** Female Lohmann Brown chicks were housed in 40 groups of 4 in wooden cages with ad libitum food and water and a 14 h photoperiod. A petri dish containing 15 drops of vanillin (4-hydroxy-3-methoxybenzaldehyde) was placed below the wire floor of each cage; this was replenished twice daily. At 8 or 9 days of age, 2 chicks were taken from each of 2 cages and the 2 pairs were placed at opposite sides of a 75 cm diameter open field (novel arena) in one of 2 test conditions, i.e., a petri dish containing either 15 drops of vanillin or a colour-matched odourless solution of food dyes was situated below the wire floor. The members of each pair were familiar to each other but strangers to the other pair; this procedure was intended to facilitate assessment of xenophobia rather than just separation-induced anxiety. The open fields were located in separate but similar rooms. One focal chick in each of the 2 pairs was marked to facilitate identification; their responses were recorded onto videotape using an overhead micro camera. We measured the latencies for the focal chicks to walk, to move to within 15 cm or less of the companion chick and to make physical contact with it. We also recorded the distance on the screen between the mid point of each chick's head at 30 s intervals during the 20 min test; the 40 measures were then summed, averaged, and translated to real life distance. GLM analysis revealed no detectable effects of day of test or day x treatment interaction so one-way ANOVA was used to examine treatment effects

**Results** The focal chick in each pair approached to within 15 cm or less of the other chick and made physical contact with it significantly sooner when the dish placed below the wire floor of the open field contained the familiar odourant (vanillin) rather than the odourless solution of food dyes (Table 1). The two chicks also remained closer together, (i.e. the distance between their heads was smaller), when the dish contained the vanillin odourant with which they had been reared rather than the food dyes.

**Table 1** Social affiliation responses (means $\pm$ standard errors) of unfamiliar chicks tested in a novel arena in the presence or absence of a familiar odourant (N=20 per treatment)

| Measure | No odourant | Odourant | P |
|---|---|---|---|
| Lat. walk (s) | 50.1 $\pm$ 13.7 | 21.8 $\pm$ 6.9 | N.S. |
| Lat 15 cm (s) | 166.5 $\pm$ 43.5 | 64.8 $\pm$ 10.5 | =0.029 |
| Lat. touch (s) | 205.5 $\pm$ 42.5 | 91.3 $\pm$ 11.7 | =0.013 |
| Distance apart (cm) | 20.2 $\pm$ 1.4 | 16.2 $\pm$ 0.5 | =0.011 |

Lat. = latency to, (s) = seconds, (cm) = centimeters, NS = not significant

**Conclusions** The presence of a familiar odourant increased social affiliation between unfamiliar chicks when two pairs of cagemates (from separate cages) were placed in a novel environment. Although chicks are xenophobic and show more aggressive pecking at strangers than cagemates at 1 day of age (Rajecki et al., 1976) inter-bird pecking was not apparent in either treatment group. Vanillin possesses no anxiolytic properties per se (Jones et al., 2001) so our findings likely reflect the reassuring nature of a familiar odour in otherwise novel surroundings. This, in turn, may have weakened any fear-induced inhibition of activity as well as reducing the chick's fear of strangers.

**Acknowledgements:** This study was supported by the UK Department of Environment, Food and Rural Affairs.

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# Effect of rearing environment on "human approach behaviour" in grower-finisher pigs.

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## Introduction

The importance of the quality of the human-animal relationship on the productivity and welfare of commercial farm animals is now well established. Previous work in pigs has found negative correlations between productivity and avoidance behaviour of a human subject (Hemsworth *et al.*, 1981). However, other reports have shown no relationship between avoidance behaviour and growth or physiological measures of chronic stress resulting from aversive handling suggesting that other factors may affect this behaviour (Pearce *et al.*, 1989). The present study examined the influence of environmental and husbandry factors on the response to humans in commercially housed grower and finisher pigs.

## Materials and Methods

On 27 breeder-finisher units detailed recordings were made of management factors such as housing, feeding and manure disposal systems; components of the physical environment such as air temperature, pen and floor types; and the social environment such as group size and stocking density. The human approach behaviour of pigs on each farm was assessed using 8 pens of grower pigs (20-45 kg liveweight) and 8 pens of finisher pigs (45-90 kg liveweight). The assessment made was based on the method of Hemsworth *et al.* (1981) which consisted of a human standing motionless at the midpoint of the wall in the dunging area of the pen and recording the time taken for a pig to approach and interact with the human. For statistical analysis, all human approach times were transformed according to the equation $y=\ln(x+0.1)$ in order to normalise the data. Forward stepwise regression models were calculated using factors giving an F-probability less than 0.25 or for continuous independent variables, a correlation coefficient of less than 0.23 ($r<0.23$ equivalent to $p<0.25$ with 26 degrees of freedom based on 27 farms).

## Results

The predictive models for grower and finisher human approach behaviour are shown in table 1. A concrete dunging area in the grower pen and an increase in the number of drinkers provided were associated with a significant increase in the human approach behaviour of grower pigs. The presence of straw in the dunging area of the grower pen and an increase in finisher pen area were associated with a significant increase in the human approach behaviour of finisher pigs. The grower model predicted 60.2 per cent of the variation in human approach behaviour ($r^2=0.602$, SE = 0.824, $p<0.001$). The finisher model predicted 50.4 per cent of variation ($r^2=0.504$, SE = 0.669, $p<0.001$).

**Table 1** *Multiple regression models for grower and finisher human approach behaviour*

| | **Grower model** | Regression coefficient | **Finisher model** | Regression coefficient |
|---|---|---|---|---|
| Constant | | 2.269[a] | | 3.849[b] |
| 1st term | Grower slatted dunging area | 1.935 | Grower slatted dunging area | -0.494 [NS] |
| 2nd term | Grower straw-bedded dunging area | 1.713 | Grower straw-bedded dunging area | -1.153 |
| 3rd term | Grower number of drinkers | -0.2895 | Finisher pen area | -0.0413 |

[a] includes Grower concrete dunging area, [b] includes Grower concrete lying area, [NS] not significant ($p>0.05$)

## Discussion

Environmental factors were significantly associated with a large percentage of the variation for both grower and finisher human approach behaviour. Floor type at the grower stage was identified as being particularly influential to both growers and finishers, although different mechanisms may be important in mediating human approach behaviour. At the grower stage it is possible that increased human contact associated with a concrete dunging area may reduce fear of humans. At the finisher stage, previous rearing with straw bedding and increased space were influential, perhaps due to the presence of a more complex environment reducing fear of humans as found by Pearce *et al.* (1989). When considering human approach behaviour as a method to evaluate stockmanship, it is important to consider effects of the current and previous rearing environments.

## Acknowledgements

The authors would like to acknowledge the financial support of the Silcock Foundation.

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# Effect of rearing environment on the prevalence of gastric ulcers in slaughter pigs.

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## Introduction

Gastric ulceration in pigs is an important condition as it has been associated with a number of signs indicative of poor welfare and reduced growth rate and FCE (Dybkjaer et al., 1994). The effects of diet, particularly feed processing methods, on the occurrence of this condition are well documented (O'Brien, 1992). It has also been suggested that "psychosomatic and other stress factors" may be important in the aetiology of this condition (O'Brien, 1992), but there is currently little information available. The present study was designed to investigate factors within the rearing environment and management techniques that were associated with the presence of gastric ulcers at slaughter.

## Materials and Methods

This study took place over a four-month period during the summer /autumn of 1998 involving 16 commercial pig units that delivered to one of three abattoirs. Detailed recordings were made of management factors such as housing, feeding and manure disposal systems; components of the physical environment such as air temperature, pen and floor types; and the social environment such as group size and stocking density were recorded by personal interview and farm visits. In addition, details of transportation to the slaughter house such as time of last feed, distance travelled, duration of the journey, weather conditions and vehicle design and layout; and of lairage conditions such as duration, pen size, stocking density and temperature were also recorded. Stomachs were collected from 50 pigs of one delivery (two deliveries if less than 50 per batch) from each farm and were scored for ulceration of the *pars oesophageal* region based on a method detailed in previous work (Elbers et al., 1995). Ulcer scores were given on a scale of 0-7, according to degree of ulceration (0= intact epithelium, 7= bleeding ulcers/erosions). For statistical analysis, forward stepwise regression models were calculated using factors giving an F-probability less than 0.25 or for continuous independent variables, a correlation coefficient of less than 0.31 ($r<0.31$ equivalent to $p<0.25$ with 15 degrees of freedom based on 16 farms).

## Results

The overall prevalence of gastric ulcers (a gastric ulcer score >3) was 19.1% with a mean ulcer score of 2.2 ± 0.15 (sem). The predictive models for gastric ulcer prevalence in slaughter pigs are shown in table 1. The predictive model explained 88 per cent of the variation in gastric ulcer prevalence ($P<0.001$). The presence of straw bedding during finishing was associated with a reduction in ulcer prevalence, whilst a concrete slatted floor during this period and a pelleted diet were associated with an increase in this disease.

**Table 1** ***Multiple regression model for factors affecting prevalence of gastric ulcers in slaughter pigs***

| Fitted Terms in Regression Model for Prevalence of Gastric Ulceration | Regression Coefficient | $r^2$ (accumulated ) |
|---|---|---|
| Constant[1] | 1.796 | |
| + Finisher slatted lying area | 0.904 | |
| + Finisher straw-bedded lying area | -0.891 | 0.62 |
| + Finisher pelleted diet | 0.951 | 0.88 |

[1] adjusted for Finisher concrete lying area

## Discussion

This study supports evidence of high prevalences of gastric ulcers in slaughter pigs in the UK (O'Brien, 1992). The current findings agree with previous studies that have reported the association of ulceration of the pars oesophageal region of the stomach and the feeding of pelleted diets (Elbers et al., 1995). This study also identifies the association between slatted floors and increased ulceration and the results suggest that the provision of straw bedding is beneficial in terms of this measure of pig welfare.

**Acknowledgements**
The authors would like to acknowledge the financial support of the Silcock Foundation.

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# Performance of newly-weaned pigs when housed with a pig with experience of creep food.

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**Introduction** When piglets are weaned at around 3 to 4 weeks of age consumption of creep food can be very variable between pigs, some not consuming any (Pajor et al, 1991), there being little opportunity to learn about solid food from the sow. Thus weaning results in a temporary reduction in nutrient intake while the pig learns to accept solid food and this results in a depression in growth, the weaning check. In other species, such as the rat, animals can learn about novel foods from a knowledgeable conspecific (Galef, 1994) but it is not clear if this transfer of knowledge occurs in pigs. Morgan et al. (2001) observed an enhanced food intake of pairs of pigs containing an experienced and inexperienced pig of the same age housed together compared to a similar pair housed apart or a pair of inexperienced pigs. Therefore, in this experiment the influence on solid food intake and growth by inexperienced piglets when housed with an experienced older pig was examined under more practical group-housing conditions.

**Materials and methods** Two hundred and forty Large White piglets were used in 10 replicates of three treatments with eight pigs per treatment group (see below). Each group of eight pigs were housed in a fully slatted pen (2.41 x 1.10m.) and the pens were situated in an artificially ventilated and heated room. Each pen had a feed bin 1.08m long x 0.25m wide with a hopper and five trough spaces. The pens also had two nipple drinkers. The piglets received no creep food until weaning. The 10 experienced pigs were weaned at 4 weeks of age, (29±0.8d and 8.6±0.53kg), one week ahead of the litter into which they were to be introduced as experienced demonstrators, and offered creep food for one week before being allocated to Treatment 1 (9.4±0.50kg). As a control, a further 10 pigs were weaned at 5 weeks (36±0.40d and 9.1±0.62kg) and allocated to Treatment 2 as inexperienced pigs of the same age as the experienced pigs. All other pigs were weaned at 4 weeks of age (27±0.36d and 7.6±0.08kg) and allocated to the treatments as follows.

Treatment 1 groups contained one experienced pig (5 week old) and seven newly-weaned pigs (4 week old) from the same litter.

Treatment 2 groups contained one inexperienced pig (5 week old) and seven newly-weaned pigs (4 week old) from the same litter.

Treatment 3 groups contained eight newly-weaned pigs (4 week old) from the same litter.

Food intake and live-weight gain were measured over a period of 14 days after grouping and were subjected to analysis of variance with the group as the experimental unit and weaning weight as a covariate.

**Results** For the 7, 7 and 8 newly weaned pigs in each group in Treatments 1, 2 and 3, respectively, daily live-weight gain was not significantly affected by treatment (Table 1). Similarly total food intake of the treatment groups (including the older pigs, i.e. 8 per group), food intake and food conversion efficiency were unaffected by treatment.

**Table 1** Live weight gain of individual inexperienced pigs, group food intake and food conversion efficiency

| Treatment | 1 | 2 | 3 | Sed | Sig |
|---|---|---|---|---|---|
| Live-weight gain g/d (14 d) | 259 | 217 | 247 | 27.8 | NS |
| Live-weight gain g/d (0 to 4 d) | 67 | 56 | 46 | 45.2 | NS |
| Group food intake kg (14 d) | 41.4 | 36.0 | 35.2 | 4.28 | NS |
| Group food conv. eff. (gain/food) | 0.80 | 0.72 | 0.76 | 0.061 | NS |

**Conclusions** The enhanced food intake recorded with paired experienced and inexperienced pigs (Morgan et al, 2001) was not evident in this experiment. Under the more variable practical conditions here the differences between treatment groups were difficult to detect statistically. Young pigs would be expected to learn from older animals but the model applied here was not sensitive enough to demonstrate this experimentally.

**Acknowledgements** The experiment was funded by the Scottish Executive Environment and Rural Affairs Department.

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# Differences in the behaviour of high and low yielding dairy cows selected by genetic merit

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**Introduction** The effects of high milk yields on the behaviour and welfare of the dairy cow are unclear. A high milk yield increases the need to consume sufficient fodder in an attempt to meet high nutrient demands. The failure to meet the demands may result in persistent hunger in the dairy cow having to modify her behaviour by employing various coping strategies. These modifications may help the cow overcome a state of hunger but at the expense of being unable to express other important behaviours. The objective of this study was therefore to determine whether the behaviour and welfare of the genetically high yielding dairy cow is being compromised by the increased nutritional demands of milk production, and to investigate the possibility that an increased amount of time spent in food-directed behaviours may have a detrimental effect on the time available to perform other important behaviours.

**Materials and methods** A group of sixty lactating, Holstein-Friesian dairy cows was initially observed over a six week period (16th April to 28th May) to record the positions of all cattle when they were lying down, predominantly feeding and entering the milking parlour, to determine which cows interacted and could not be considered independent. Forty cows were selected that were not observed to interact and were divided into two groups of 20 cows, high-yielding and low-yielding, with mean yields of 40.6 kg/d (s.e. 0.51) and 31.0 kg/d (s.e. 0.75) respectively. The low-yielding cows had increased mean body condition score of 3.10 (s.e. 0.06), compared with 2.80 (s.e. 0.04) for the high-yielding cows, and they were, on average, 238 d (s.e. 9.27) into lactation, compared with 204 d (s.e. 10.9) for high-yielding cows. Both groups were kept together as part of a herd of 60 cows, and were housed indoors between 14.15h and 08.30h in a large straw-bedded shed with *ad libitum* access to drinking water and a total mixed ration. For the rest of the day (08.30h to 14.15h), the cows grazed in a 4.74 ha paddock, with access to drinking water. Behaviour was recorded by three observers over a 48h period. Sixteen mutually exclusive behaviours (total feeding, drinking, lying, lying ruminating, standing ruminating, sleeping, standing, walking, grooming self (lying or standing), grooming others (lying or standing), mouthing housing furniture, nosing housing furniture, rubbing housing furniture and vocalising) were recorded for each cow using instantaneous scan sampling (Martin & Bateson, 1995) at 20 minute intervals whilst outside and at 10 minute intervals during the indoor period. The distribution of all behaviours was normal, and were analysed statistically by ANOVA, except those behaviours classified as stereotyped, which were analysed by a non-parametric test (Mann-Whitney). The total time spent standing (grooming others standing, grooming self standing, ruminating standing, standing, and walking) and lying (grooming self lying, grooming others lying, lying, ruminating lying, and sleeping) and in stereotyped behaviours (mouthing, nosing, rubbing, and grooming self) were analysed separately.

**Results** High yielding cows spent more time feeding and standing, but less time lying and resting, compared with low yielding cows. The median number of sterotypies was less for high yielding cows than low yielding cows. No significant ($P > 0.05$) differences were observed for the following behaviours; drinking, lying, lying ruminating sleeping, standing idling, walking.

**Table 1** Frequency of observations during a 48 h period for the low and high yielding cows

| Behaviour | Mean ± s.e | | *P* – Value |
|---|---|---|---|
| | Low yielding | High yielding | |
| Total feeding | 36.0 ± 1.25 | 40.2 ± 1.34 | 0.030 |
| Ruminating standing | 5.2 ± 0.43 | 7.7 ± 0.45 | 0.076 |
| All standing behaviours combined | 23.6 ± 1.92 | 29.9 ± 2.43 | 0.048 |
| All lying behaviours combined | 127.2 ± 2.24 | 117.6 ± 2.94 | 0.014 |
| Median total stereotypic behaviours | 2.0 | 1.0 | 0.028 |

The following behaviours were significantly ($P \leq 0.05$) positively correlated with milk yield; feeding (0.348), ruminating standing (0.312), and combined standing (0.315). Combined lying behaviours were significantly ($P \leq 0.05$) negatively correlated (-0.398) with milk yield.

**Conclusions** High yielding cows spend more time feeding and standing and less time lying and resting. As cattle display a strong motivation to lie down (Metz, 1985), any reduction in resting behaviours can be considered detrimental to the cows' welfare. A reduction in stereotyped behaviours in high yielding cows may have been due to greater feeding activity.

**Acknowledgements** Moulton College for a studentship for M. Cooper. This work was supported by a research grant from the University Federation of Animal Welfare.

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# Factors affecting neonatal viability and the relationship between it and subsequent creep feeding behaviour of suckling piglets

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**Introduction** Neonatal viability is one of the key factors affecting piglets' vitality, which ultimately affects the survival and growth of piglets (England, 1974). As colostrum is the only food resource of neonatal piglets, their ability to acquire the colostrum as early as possible after their birth can determine their vitality. Piglets are usually supplied with creep food at some time during the suckling period in order to improve their performance before and after weaning. However, the creep food intake varies between litters and between individuals. Furthermore, the relationship between viability in early life and the acceptance of a new food (e.g. creep food) when they first encounter it, is not fully understood. The objectives of this study were to investigate factors affecting the neonatal viability of piglets at birth and to identify the relationship between neonatal viability and subsequent creep feeding behaviour by piglets on d14-d15.

**Materials and methods** A total of 101 Large White x Landrace piglets from 8 litters were used in this study. After birth, each piglet was marked with a large number on its back for video recording. The neonatal viability in terms of latency of the first suck and the number of sucks of each piglet 8 hours after birth was analysed from the videotape. A feeder containing solid food was supplied to the kennel area from d14. Piglets' creep feeding behaviour was video recorded for 24 hours on d14-d15. Data were analysed in GENSTAT using REML. The fixed model specified in the analyses included the parity of sows, sex, birth weight and birth order of the piglet. Piglet within litter was considered as the block factor in the random model. The relationships between the neonatal viability and the creep feeding behaviour on d14-15 was analysed by Pearson correlation on the basis of the eight litter means.

**Results** The results showed that piglets with higher birth weight had a higher cumulative number of the incidence of suck ($P<0.01$) though the latency of the first suck after birth was not affected by birth weight) (Table 1). On d14, when creep food was introduced the lighter piglets at birth tended to contact the food slower ($P<0.10$). However, the cumulative number of times piglets touched food was not affected by birth weight of piglets over 24 hours on d14-15. Neither sex nor birth order affected the neonatal viability and the creep feeding behaviour on d14-15. There were no clear correlations between neonatal viability and creep feeding behaviour (Table 2).

**Table 1** The effect of sex, birth order (BO) and birth weight (BW) of piglets on neonatal viability and creep feeding behaviour on d14-15. No sex and birth order effects were significant. + = $P<0.10$, ** = $P < 0.01$.

| | | Wald statistics* | | |
|---|---|---|---|---|
| Variable | mean | Sex | BO | BW |
| Latency of the first suck after birth, min | 50.4 | 0.0 | 4.2 | 6.1 |
| Cumulative number of sucks 8 h after birth, no/8h | 198.3 | 0.3 | 4.1 | 19.2** |
| Latency to contact creep food on d14, min | 70.6 | 2.6 | 3.9 | 7.8+ |
| Cumulative incidence of touching food on d14-15, no/24h | 0.9 | 2.1 | 1.6 | 1.9 |

*tested against Chi-squared distribution (1df) to give the significance of the term in the model

**Table 2** Correlation coefficient of neonatal viability after birth and creep feeding behaviour on d14-15.

| | Latency of the first suck after birth | Cumulative number of sucks 8 h after birth |
|---|---|---|
| Latency to contact creep food | -0.592 | -0.287 |
| Cumulative incidence of touching food | 0.642+ | -0.535 |

**Conclusions** The results suggest that neonatal viability in terms of cumulative suck number was correlated to birth weight rather than sex and birth order. Live weight tended to correlate to creep feeding behaviour in terms of the latency to contact the creep food. However, the relationship between the neonatal viability and creep feeding was not clear in the present study.

**Acknowledgement** The authors acknowledge the assistance of colleagues at Easter Howgate Farm, Edinburgh. H. F. Lee also wishes to thank the Ministry of Education of Taiwan for provision of a PhD Studentship.

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# Interactions between behavioural development, plasma cortisol and thermoregulation in the neonatal lamb

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**Introduction** The behavioural development of precocious mammals contributes to their survival in the neonatal period. In the sheep, neonatal behavioural progress is known to be affected by breed and birthweight (Dwyer, 2001), and is related to lamb survival (Dwyer et al., 2001). Lambs that are slow to stand and suck may be less mature at birth than lambs that stand quickly. Foetal cortisol plays an important role in the preparation of the lamb for postnatal life (Liggins, 1994) and may also play a role in the development of behavioural competency at birth. In this study differences in behavioural develoment and plasma cortisol were measured in two breeds of sheep known to differ in behavioural competency at birth. In addition, ability to maintain body temperature was also investigated.

**Materials and Methods** Data were collected from 78 neonatal lambs of two breeds (42 Blackface, 36 Suffolk) born as singles (n=19), twins (n=50) and triplets (n=9). For each lamb the time from birth to first stand and first suck were recorded. In addition, rectal temperatures were taken between 30 and 60 mins of birth (mean = 45.7 ± 1.6), and at 24 and 72 h of age. Blood samples were also collected by jugular venepuncture at the same time as the temperature measurements. Plasma from these samples were analysed for cortisol. Birth weight of the lamb was determined at approximately 24 hours of age. The effects of breed, birth weight and behaviour on body temperature and plasma cortisol were determined by the Restricted Maximum Likelihood Procedure (REML, Genstat) or by non-parametric tests (Kruskal Wallis ANOVA) in data that were not normally distributed.

**Results** Suffolk lambs were significantly heavier than Blackface lambs (mean birth weight (kg): Suffolk = 4.91 ± 0.21, Blackface = 4.19 ± 0.1, P<0.01) and had a signficantly longer gestation (median gestation length (days): Suffolk = 145.8, Blackface = 143.5, *H*=18.95, d.f.=1, P<0.001). Suffolk lambs were slower to stand and fewer sucked within 2 hours of birth than Blackface lambs (proportion sucking in 2 h: Suffolk = 0.41, Blackface = 0.81, $\chi^2$=17.4, d.f.=3, P<0.001). Rectal temperature was higher in Blackface lambs than Suffolk lambs at all ages (Table 1) and plasma cortisol was lower when averaged over the whole period (P<0.01; Table 1). When all lambs were considered body temperature and time to stand and suck were related (Table 2). This relationship was also evident within breed although this was significant only for the Blackface lambs (P<0.05). In Suffolk lambs, but not Blackface, body temperature was positively correlated with birth weight (e.g. temperature at 24 h, $r_s$=0.38, P<0.05) and plasma cortisol negatively correlated with birth weight ($r_s$=-0.35, P<0.05). Cortisol also tended to be higher in lambs that were slow to suck (P=0.08). Cortisol and rectal temperature were negatively correlated in all lambs at <1 h of age ($r_s$=-0.5504, P<0.001). By 24 h, cortisol and temperature were negatively correlated in Suffolk lambs only ($r_s$=-0.38, P<0.05).

**Table 1:** *Effect of breed on temperature and cortisol*

| Temp °C | < 1h | 24 h | 72 h |
|---|---|---|---|
| Blackface | 39.92 | 39.47 | 39.54 |
| | (39.3-40.3) | (39.1-39.6) | (39.4-39.8) |
| Suffolk | 38.68 | 38.89 | 39.17 |
| | (37.9-39.7) | (38.2-39.2) | (38.9-39.6) |
| | P<0.001 | P<0.001 | P<0.001 |
| Cortisol (ng/ml) | | | |
| Blackface | 119.1 | 39.71 | 27.67 |
| | (88.5-164) | (29.9-52.8) | (15.7-42.4) |
| Suffolk | 149.2 | 63.97 | 32.51 |
| | (85.1-205) | (48.1-85.1) | (18.6-51.3) |
| | NS | P<0.01 | NS |

**Table 2***: Effect of temperature on lamb behaviour*

| Stand | Temp (°C) | Suck | Temp (°C) |
|---|---|---|---|
| <30 min | 39.38 | < 1 h | 40.16 |
| | (38.6-40.2) | | (39.5-40.4) |
| 30-60 min | 38.80 | 1 – 2 h | 39.66 |
| | (38.0-39.8) | | (38.6-40.0) |
| 60-90 min | 37.88 | 2 – 3 h | 39.26 |
| | (36.8-38.9) | | (38.3-40.0) |
| 90-120 min | 37.13 | 3+ h | 39.00 |
| | (36.2-38.0) | | (37.6-39.9) |
| | *H*=9.98, df=3, P<0.05 | | *H*=13.7, df=3, P<0.01 |

**Conclusions** Despite their longer gestation length, Suffolk lambs appeared less mature at birth – they took longer to stand and suck and were less able to maintain body temperature than Blackface lambs. Birthweight, plasma cortisol and body temperature were more closely related in Suffolk lambs than Blackface. The quicker time to stand and suck in the Blackface may be partly a function of their greater ability to maintain body temperature.

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# Influence of salt application on biting by growing-finishing pigs.

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**Introduction**. In intensively kept fattening pigs, biting is a vice complex of fairly common occurrence, of widespread distribution, of some economic importance but of obscure aetiology. It has been suggested that in any one particular outbreak of biting there may be more than one aetiological factor at work and that the behaviour may only occur when the sum of the individual effects of these factors goes beyond a certain critical (undefined) level (Ewbank, 1973). Factors such as: sub-optimal space allowance, temperature control, air movement, nutrients, palatability of feed, access to feed and water, can stimulate this kind of behaviour. Salt (NaCl) is an essential mineral and its rate of inclusion in diets has been implicated in the development and expression of biting behaviour. It is possible that heightened appetite for salt could make pigs particularly attracted to pen-mates with injured tails (Fraser, 1987). Although pigs require only about 0.2% NaCl in the diet for maximum weight gain, NaCl is often provided at 0.5% of the diet for growing pigs, and it has been suggested that an increase to 0.75% or 1% can reduce the incidence of biting. This paper investigates the influence of salt application on biting by growing-finishing pigs under an intensive indoor pig production system. An amount of extra salt was provided to the pigs and behavioural observations (biting, drinking, lying and standing) were recorded.

**Materials and methods.** The effect of providing pigs with additional salt was investigated as a complete randomised design. Four pens of pigs were studied on a commercial pig unit. The pens were bedded with straw, and were stocked with 90 female pigs (50% Large White, 25% Duroc, 25% Landrace) between 53-75 kg liveweight, at a stocking density of 0.5m$^2$ pig. Two pens (replicates) of pigs served as the controls and received feed containing 0.5% salt. Pigs in the two treatment pens received additional salt (equivalent to 1% extra NaCl per kg feed). Salt applications (1 kg) took place twice daily with a three hour interval between applications. The salt was distributed at several places on the pen floor to ensure that the majority of the pigs had a chance to eat some of it. The additional salt was given three times per week (Monday-Wednesday-Friday). The pigs were observed once per hour for any kind of behavioural responses (drinking, biting, lying or standing) from 9 am to 5 pm on the given days for a period of one month. Care was taken to ensure that the water supply was adequate throughout the experiment. The data was analysed using One Way Analysis of Variance.

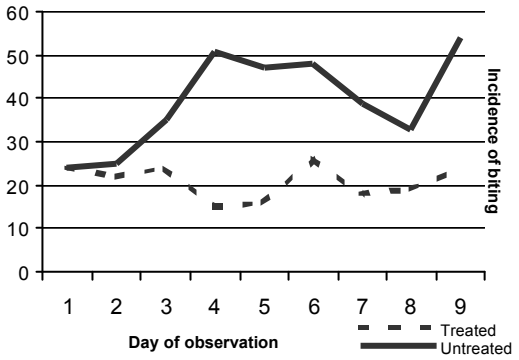
**Results.**

**Table 1** Effect of providing additional salt on the incidence of biting by growing finishing pigs.

| Observations. (Mean no. of animals) | Control (0.5% diet) | Additional salt[a] | SED |
|---|---|---|---|
| Bites | 170.5 | 77.5 | 13.51* |
| Drinking | 130 | 127 | 2.12 |
| Standing | 187.5 | 199 | 8.13 |
| Feeding | 357 | 338.5 | 13.08 |
| Lying | 3958.5 | 3937.5 | 14.85 |
| % of Total Bites. | 68.75% | 31.25% | |

[a] Additional salt equivalent to 1.5% diet.
* Statistically different (P<0.02)

**Figure 1** Incidence of biting on the different treatments.




There was no significant difference between the treatments for behaviour measurements other than bites. On average, less than half as many bites (P<0.02) were recorded in the pens that received additional salt compared with the untreated pigs (Table 1). There were consistent differences between the two treatments on each of the days when observations were conducted (Figure 1).

**Conclusions:** The results from this study support the view that the provision of additional salt can reduce the incidence of biting by growing-finishing pigs. It is suggested that before other factors are considered, salt content of the diet should be checked. Providing that pigs have ready access to water, increasing their salt intake may improve their behaviour.

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# Sham dustbathing and use of dustbaths in furnished cages for laying hens

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**Introduction** The furnished cage for laying hens is one attempt to meet the welfare problem of behavioural restriction in conventional battery cages. However despite the presence of a dustbath, sham dustbathing on the cage floor, which is usually associated with litter deprivation, is often seen. It has been suggested that the performance of sham dustbathing is satisfying enough to substitute for dustbathing in litter (Lindberg & Nicol, 1997) but later experimental studies have failed to show that it reduces the motivation to dustbathe in litter (Lindberg, 1999; Olsson *et al*, accepted). An alternative hypothesis is that sham dustbathing is a consequence of social competition since the dustbath is not big enough to allow all birds in a cage to use it at the same time. If this hypothesis is true, sham dustbathing would be expected to take place mainly when the dustbath is occupied. We tested the hypothesis by observing sham dustbathing and the use of dustbaths in furnished cages.

**Materials and methods** Cage-reared LSL laying hens (N=5000) were kept in conventional and in furnished cages on a commercial farm. Both types of cages were in the same room in a three-tier system, with two back-to-back rows of each cage type (Conventional cages: N=648, 3 hens per cage, 714 $cm^2$ floor space per hen; Furnished cages: N=348, 7 hens per cage, 858 $cm^2$ floor space per hen). The furnished cages were equipped with a perch, a nest at floor level and a dustbath (with woodshavings) situated on top of the nest box. There were two types of furnished cages, differing in the design of the entrance to the dustbath: narrow-entrance cages (N=174) with a dustbath entrance (140 x 180 mm) where the door slid upwards and sideways at opening and wide-entrance cages (N=174) with a dustbath entrance (200 x 180 mm) with a top-hinged door opening into the cage. The dustbaths were first opened when birds were 21 weeks old and were thereafter opened in the afternoon between 14.40 and 17.10 h. The hens were observed during four days, with each cage being observed at least two times per day. Number of hens sham dustbathing and location of sham dustbathing was recorded as well as whether or not the dustbath was open, number of hens in the dustbath and number of hens dustbathing. On the first day, observations were made when the dustbaths were open between 14.40 and 17.00 h. Since it was found that very few hens sham dustbathed at this late hour, it was decided to observe hens earlier in the day, both when the dustbaths were opened at the normal time and when they were opened earlier. Therefore, on the second and third days, observations were made between 10.15 and 14.40 h before the dustbaths opened. On the fourth day, the dustbaths were opened at 8.30 h and observations were carried out between 10.15 and 14.40 h.

**Results** Birds were seen sham dustbathing in all three types of cages, and in the furnished cages sham dustbathing was seen in all treatments, irrespective of whether and when the dustbaths were opened. Most sham dustbathing took place where birds could reach a dusty substrate for pecking. Sham dustbathing rarely coincided with the dustbath being occupied; in fact, in the majority of observations when the dustbath was open, it was not occupied. When the dustbath was not available, the amount of sham dustbathing was similar in the conventional and the furnished cages (Table 1).

**Table 1**. Behaviour when the dustbaths were not available, indicated as number of hens (percentage of observations).

| | Conven-tional | Modified narrow | Modified wide |
|---|---|---|---|
| Sham dustbathing | 28 (1.1) | 24 (0.79) | 39 (1.3) |

Type of dustbath entrance affected both number of birds in the dustbath and number of birds dustbathing, but had less effect on number of birds sham dustbathing, as can be seen in Tables 2 and 3.

**Table 2**. Behaviour when the dustbaths were opened at normal time, indicated as number of hens (percentage of observations). Statistics calculated using odds ratio.

| | Modified narrow | Modified wide | *P* |
|---|---|---|---|
| Sham dustbathing | 4 (0.30) | 2 (0.13) | *ns* |
| In the dustbath | 22 (1.6) | 91 (5.8) | *0.0001* |
| Dustbathing | 10 (0.74) | 46 (2.9) | *0.0001* |

**Table 3**. Behaviour when the dustbaths were opened early, indicated as number of hens (percentage of observations). Statistics calculated using odds ratio.

| | Modified narrow | Modified wide | *P* |
|---|---|---|---|
| Sham dustbathing | 47 (1.3) | 31 (0.85) | *0.07* |
| In the dustbath | 25 (0.68) | 120 (3.3) | *0.0001* |
| Dustbathing | 14 (0.38) | 45 (1.2) | *0.0001* |

**Conclusions** There was no evidence from this study thatproviding birds with a dustbath in a furnished cage decreased sham dustbathing. We suggest that sham dustbathing in furnished cages may be related to the dustbath opening late at the day as well as the effect of early experience as most layer pullets are reared without access to litter.

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# Excretory behaviour of lactating sows in an outdoor organic production system

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**Introduction** The excretory behaviour of outdoor lactating sows has important implications for sow and piglet health, especially in organic systems, where use of anthelmintics and other medication is restricted. It is also important in determining the environmental impact of the system. If foraging and excretion are spatially separated this limits risk of parasite infection, but may lead to nutrient "hotspot" formation with potential for leaching and poor nutrient cycling to subsequent crops. Where nose-ringing of organic sows is not permitted by the certification scheme, pasture will be destroyed by foraging activity, further promoting nutrient losses. This study aimed to investigate the spatial distribution of excretory behaviour and patterns of pasture loss during the period from farrowing to weaning.

**Materials and methods** Paddocks for individual lactating sows were arranged in two rows of five, each approx 20 x 20 m, on a second-year grass-clover ley. Sows were fed in two discrete meals daily according to body condition and had *ad libitum* access to water from a trough. The sows were moved into the block shortly before farrowing, and remained in the same paddock during Feb-April 2001 until all sows were weaned on the same day (7-10 weeks after farrowing). To record excretory behaviour, the paddocks were notionally divided into ten locations (Fig 1). The location of each defaecation or urination in all paddocks was recorded, from approx 08:00-12:00 and 13:30-17:30 on up to three days in each week after farrowing. The % vegetation cover in each location was subjectively assessed in each week. The frequency of excretory behaviour in each location was compared with that expected randomly on the basis of relative area using chi-square tests. Further analysis was conducted by combining locations into functional regions as follows: locations 1, 2, 3, 100 (beside hut) were combined as "feeding"; locations 4, 6, 8, 9 were bordered by other sows ("pig borders"), except for the four sows at the ends of the block , where location 6 bordered a trackway ("non-pig borders"); location 7 also bordered other sows, but contained the water, and so was considered separately in the functional regions ("drinking"). Area 5 (51+52) remained as a "central" region.

| | | | |
|---|---|---|---|
| 7 | 8 | | 9 |
| | | 52 | |
| 4 | 51 | 100 hut | 6 |
| 1 | 2 | | 3 |

Fig 1. Plan of lactating sow paddock showing locations

**Results** Sows showed a distinct location preference in their excretory behaviour (Fig 2). Locations 1, 2, 3, 51, 52 were significantly underutilised, and location 8 was utilised significantly more than expected ($p<0.05$). When the functional regions were considered, "pig borders" and "drinking" were utilised significantly more than expected ($p<0.05$), but none of the other regions differed from expectation. Defaecation and urination followed the same pattern in the functional areas. The feeding and drinking areas were denuded of grass first (3% green cover after 6 weeks), with the rest of the paddock retaining some grass cover for the majority of the lactation period (pig borders 26% green at 6 weeks) (Fig 3).

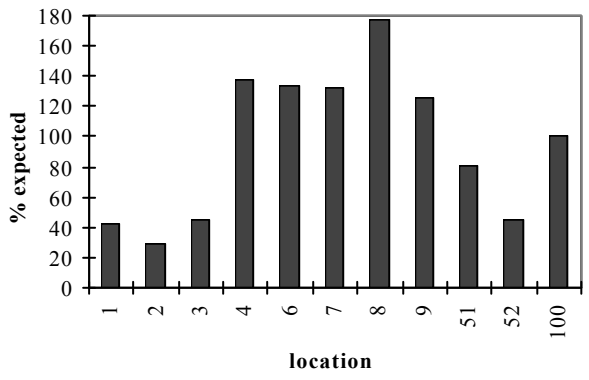


Fig 2. Excretory behaviour of lactating sows at different paddock locations (observed as % expected)

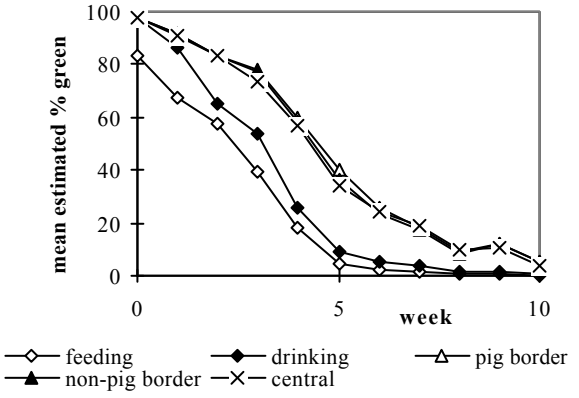


Fig 3. Mean estimated % vegetation cover in functional areas of lactating sow paddocks up to 10 weeks occupancy

**Discussion and conclusions** These data show that the sows performed more excretory behaviour at the pig borders, but showed no other preference, suggesting that territorial behaviour was their primary motivation. The decline in grass cover indicates that the pigs were foraging in all areas. Even a low level of foraging behaviour in a favoured defaecation area may be sufficient for infection with endoparasites or other pathogens. The study was conducted at the end of an exceptionally wet winter (rainfall Feb-Apr 2001 285.5 mm, 10 year av 183.5 mm). It is possible that during a drier period, more of the grass cover would have been retained. However, the combination of high rainfall, low vegetation cover and localised excretion gives high pollution potential from leaching. Wasted feed may lead to high nutrient levels in the feeding area where there was little grass remaining for retention.

**Acknowledgements** This study was linked to a project on optimising organic pig production funded by DEFRA.

# Effect of tooth clipping on piglet behaviour

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**Introduction** Piglets with intact teeth suffer considerable damage to the skin of the face during establishment of the suckling hierarchy (Delbor et al., 2000). However, the direct and indirect damage caused by clipping can exceed the effects of these injuries (Noonan et al., 1994; Hutter et al., 1994). The objective of this study was to compare the behaviour of piglets with intact or clipped needle teeth and to discuss the welfare implications of any differences observed.

**Materials and methods** Litters of twenty-four Large White x Landrace post-parturient sows were assigned to either intact (I) or clipped (C) treatments on the basis of litter size. After birth all piglets had their ears notched and tails docked, in addition the needle teeth of all piglets in 12 litters were clipped. Piglets in the remaining 12 litters were returned to the farrowing crate with their teeth intact. One boar and one gilt in each litter were selected as focal animals on the basis of being nearest to the average weight of the litter when piglets were weighed individually 5, 10 and 15 days post-partum. On these days focal animals were directly observed twice between 1400 and 1700h. Observation periods for both treatments were matched in time, pen order and gender of the focal animal. Individual observation periods lasted 5 minutes, during which time the behaviour of the focal animal was recorded continuously onto a Psion organiser. Behaviour was compared between treatments using the Mann Whitney U-test.

**Results** Selected behavioural parameters are presented in Table 1. Piglets with intact teeth spent more time walking/running and engaged in individual and social play on day 5. They also tended to spend more time exploring ($P<0.06$). Similarly, piglets with intact teeth performed more individual play and spent more time walking/running than piglets with clipped teeth on day 15. They also spent significantly more time exploring and engaged in 'agonistic' play on this day. Piglets with clipped teeth spent longer sleeping than piglets with intact teeth on day 15. There were no significant differences between treatments on day 10 ($P>0.05$).

**Table 1** *Median (min.-max.) duration (% of observation time) of different behaviours performed by piglets with clipped (C) and intact (I) needle teeth on three observation days*

| | Day 5 | | Day 10 | | Day 15 | |
|---|---|---|---|---|---|---|
| | C | I | C | I | C | I |
| Sleep | 44.8 (0-67.1) | 45.6 (0.5-53.4) | 37.4 (0-50.0) | 45.3 (0-50.6) | 50.8 (2.3-64.3) $^{x}$ | 14.9 (0-45.9) $^{y}$ |
| Explore | 0.8 (0-4.9) | 3.6 (0-11.2) | 1.9 (0-7.8) | 0.8 (0-8.0) | 1.2 (0-5.2) $^{c}$ | 9.8 (0-18.9) $^{d}$ |
| Individual play | 0 $^{a}$ | 0.5 (0-1.2) $^{b}$ | 0 | 0 | 0 $^{a}$ | 0 (0-0.4) $^{b}$ |
| Agonistic play | 0 | 0 (0-1.4) | 0 (0-0.4) | 0 (0-1.0) | 0 (0-0.4) $^{a}$ | 1.1 (0-1.8) $^{b}$ |
| Social play | 0.1 (0-2.0) $^{c}$ | 2.2 (0-7.0) $^{d}$ | 0.3 (0-3.7) | 0.6 (0-2.6) | 0 (0-5.3) | 2.4 (0-4.7) |
| Walk/run | 3.6 (0-5.9) $^{a}$ | 5.6 (0-16.0) $^{b}$ | 3.1 (0-5.5) | 3.8 (0-9.8) | 1.7 (0-3.5) $^{x}$ | 8.1 (0-15.0) $^{y}$ |

$^{a,b}$ $P<0.05$; $^{c,d}$ $P<0.01$; $^{x,y}$ $P<0.001$

**Conclusions**. The reduction in play behaviour brought about by teeth clipping indicates that the welfare of these piglets was poorer than that of piglets with intact teeth (Lawrence, 1987). Indeed, piglets with clipped teeth spent less time performing exploratory and social play behaviours which could reflect pain caused by traumatic injuries in the mouth as these behaviours involve considerable use of the head and snout. They also spent less time locomotory and engaged in playful fighting, behaviours that are negatively correlated with physiological measures of stress in piglets (Worsaae and Schmidt, 1980). Finally, 15-day-old piglets with clipped teeth spent longer sleeping which suggests that they may have been suffering from the effects of, or recuperating from, stress or infection (Rampin et al., 1991; Toth et al., 1996). The behavioural differences between the two treatments indicate that clipping the incisors of newborn piglets has negative health and welfare implications for young piglets.

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# Effect of floor type on the welfare of piglets in the farrowing house

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**Introduction** Floor type is one of the main features influencing the welfare of sows and piglets in farrowing crates. Yet it is difficult to reconcile the needs of the sow and her piglets through the use of one floor (Furniss et al., 1986). Hence the aim of this study was to identify a floor combination that optimises the welfare of the piglets in the farrowing crate.

**Materials and Methods** Five days prior to entering the farrowing house, 63 Large White x Landrace sows were assigned on the basis of parity to farrowing crates containing one of four different floor combinations (Table 1).

Table 1. Floor treatments

| Floor under sow | Floor under piglet | Treatment name |
|---|---|---|
| Tri-bar$^{R}$ | Tri-bar$^{R}$ | TT |
| Tri-bar$^{R}$ | Super-coated$^{R}$ | TS |
| Cast iron | Super-coated$^{R}$ | CS |
| Super-coated$^{R}$ (plastic coated expanded metal) | Super-coated$^{R}$ | SS |

Piglets were inspected for injuries to the body and feet, which were scored according to severity, by a scoring method adapted from Penny et al. (1963) 24 hours after birth (Day 1), at 8 days of age (Day 8) and at 15 days of age (Day 15). Mean litter skin and foot lesion scores were calculated. A two-hour scan sample of piglet behaviour was carried out between 24 and 48 hours after birth. Analysis was carried out using the SAS package. Repeated measures ANOVA was used to analyse litter lesion scores. Scan samples were analysed using the Kruskall-Wallis test.

**Results and Discussion** On each inspection day piglets on the TT floor had significantly higher skin and foot lesion scores than piglets on the other three floors (Table 2). There were no significant differences between the other three treatments ($P > 0.05$).

Table 2. Mean (± SE) Skin and Foot Lesion Scores

| | Skin Lesion Scores | | | | | Foot Lesion Scores | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Floor** | **TT** | **TS** | **CS** | **SS** | **P value** | **TT** | **TS** | **CS** | **SS** | **P value** |
| **Day1** | $1.3^{a}$ ± 0.18 | $0.5^{b}$ ± 0.12 | $0.5^{b}$ ± 0.15 | $0.5^{b}$ ± 0.15 | 0.001 | $24.4^{a}$ ± 1.16 | $18.2^{b}$ ± 1.20 | $14.9^{b}$ ± 1.31 | $16.3^{b}$ ± 1.73 | 0.001 |
| **Day8** | $2.1^{a}$ ± 0.24 | $1.1^{b}$ ± 0.18 | $1.3^{b}$ ± 0.27 | $1.0^{b}$ ± 0.29 | 0.01 | $22.4^{a}$ ± 0.96 | $17.1^{b}$ ± 1.47 | $16.9^{b}$ ± 1.83 | $17.0^{b}$ ± 1.57 | 0.01 |
| **Day15** | $2.5^{a}$ ± 0.20 | $1.4^{b}$ ± 0.24 | $1.5^{b}$ ± 0.27 | $1.3^{b}$ ± 0.15 | 0.001 | $20.1^{a}$ ± 1.14 | $12.2^{b}$ ± 1.09 | $10.5^{b}$ ± 1.47 | $12.4^{b}$ ± 1.28 | 0.001 |

$^{a,b}$Different superscripts across rows indicate significant differences ($P < 0.05$)

The proportion of observations in which piglets were seen lying on the heat pad was significantly higher on the TT and TS floors compared to the SS floor (Table 3). There were no other significant differences in piglets' use of different areas in the crate on different treatments.

Table 3. Percentage of observations (± SE) in which piglets were observed in different areas of the crate

| | TT | TS | CS | SS | P value |
|---|---|---|---|---|---|
| **Active at Teats** | 23.9 ± 1.8 | 24.5 ± 2.3 | 19.8 ± 2.6 | 23.4 ± 3.0 | NS |
| **Active on Heat Pad** | $8.0^{b}$ ± 0.9 | $9.4^{b}$ ± 0.9 | $7.9^{b}$ ± 1.8 | $4.3^{a}$ ± 0.7 | 0.05 |
| **Inactive on Heat Pad** | 57.6 ± 2.1 | 52.4 ± 2.7 | 58.2 ± 2.0 | 56.9 ± 4.4 | NS |
| **Other areas of pen** | 8.8 ± 1.7 | 12.1 ± 1.8 | 13.4 ± 2.7 | 16.6 ± 4.2 | NS |
| **Area under sow** | 1.3 ± 0.6 | 0.5 ± 0.3 | 0.8 ± 0.3 | 0.5 ± 0.3 | NS |

$^{a,b}$Different superscripts across rows indicate significant differences ($P < 0.05$)

**Conclusions** Combination floors (TS, CS, SS) gave rise to less severe injuries than the TT floor, and so are positive from a piglet welfare perspective. There was no difference between the floors used in conjunction with Super-coated$^{R}$, indicating that it is the floor in the piglet area that is critical in terms of piglet welfare. However it is desirable that piglets utilise the heat pad and as a result the SS treatment may be less suitable in the farrowing crate than the other two combination floor treatments (CS, TS).

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# Does the experience of the stock-person alter the behaviour of the ewe during handling and management?

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**Introduction** The recent trend within the Agricultural Livestock Industry away from employing permanent staff to utilising casual staff as a result of a decline in profitability may have implications for the welfare of livestock. This factor has not been examined in detail even though there are a number of studies concerned with the role of the stockperson (for example, Duncan and Fraser, 1993). From the limited number of studies performed, it is likely that the interaction between the stock-person and the animal is negative as adverse behavioural patterns are identifiable, even if the stock-person is experienced. The study reported compares the behaviour of ewes being managed by an experienced stock-person or by students undergoing training.

**Materials and method** Close circuit television (CCTV) and time lapse video recording (VCR) were used to observe the behaviour of the ewes (n=16) with the experienced stock person known to the animals (E) and with students (n=10) undergoing training (T). Both the stock person and the students involved in the study were not aware of the actual time and date when observations were made. The behaviour of the stock-person and the ewes were recorded, continuously (10 to 15 minute sampling period) using an ethogram, even when the pens were passed and not entered. Initial behavioural patterns of the ewe when the stock-person passed the pen were classified into 6 classes, ewe turns to face human (FH), head turn but no body position change (HT), remain eating (RE), remain lying (RL), remain not facing human (BT) and no apparent change in behaviour (NC). When the stock person entered the pen, the initial behavioural responses were classified into 3 classes; approach the stock-person (AP), no movement (NM) and move away or flight (MA). Flight distance was measured if the ewe moved away. The behavioural changes were analysed using chi square and flight distances using the single observation *t*-test (Sokal and Rohlf, 1981).

**Results** The responses of the ewe to the in-experienced stock-person can be clearly identified even if the human does not enter the pen the animal is housed in. The main behavioural changes observed were an increase in the incidence of the ewe turning to face the inexperienced stock-person (FH; $P<0.01$) and an increase in the ewe moving away from the inexperienced stock-person (BT; $P<0.01$). When the experienced stock-person was present, ewes tended not to change behaviour from the previous pattern recorded (NC; $P<0.01$, HT, RE and RL no significant difference; Table 1).

**Table 1** Behaviour of ewes when stock-person passes the pen but does not enter (proportion of observations showing animal behavioural response).

| | E | T | Significance |
|---|---|---|---|
| FH | 0.127 | 0.246 | ** |
| HT | 0.242 | 0.278 | NS |
| RE | 0.082 | 0.045 | NS |
| RL | 0.151 | 0.108 | NS |
| BT | 0.167 | 0.255 | ** |
| NC | 0.231 | 0.068 | ** |

When the pen was entered by either stock-person the flight distance and behaviour of ewes were recorded. In a small proportion of cases, the ewe approached the experienced stock-person (0.095; AP), whereas with the inexperienced stock-person, the animal rarely approached (0.027). Flight response of ewes was markedly increased ($P<0.05$) when the inexperienced entered the pen (2.53 m s.e. 0.4) compared to the distance moved when the experienced stock-person entered the pen (1.14 m s.e. 0.6) .

**Conclusion** Handling and management are important factors affecting the welfare of the animal. In-experienced stock-persons may disrupt the behaviour of the animal leading to an increase in stress. However when training stock-persons at undergraduate level, handling of animals is important in developing an understanding of the behaviour and welfare of the species.

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## Leptin and its receptors: Modulation of the neuroendocrine axis

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Leptin is a 16 kDa protein synthesized and secreted primarily by adipocytes. Leptin was discovered in 1994 as the gene product deficient in the obese *ob/ob* mouse. In addition to profound obesity, these animals are characterized by multiple, complex neuroendocrine disorders that manifest in severe insulin resistance and infertility. We now know that leptin is involved in the regulation of appetite, body weight, energy balance, reproduction and the neuroendocrine axis in animals and man. Additionally, leptin has been shown to regulate immune function and anorexia associated with disease.

Leptin elicits its myriad effects via binding to, and activation of, leptin receptors. Leptin receptors belong to the class 1 cytokine receptor family. Multiple leptin isoforms exist due to alternative splicing. The long form of the leptin receptor is highly expressed in key hypothalamic feeding centers as well as in the pituitary. Rodents with mutations in the leptin receptor gene (db/db mice, fa/fa rats) are leptin resistant, hyperphagic and obese. Although the signaling pathways used by the long form leptin receptor are beginning to be elucidated, the physiological role(s) of truncated leptin receptor isoforms expressed in many tissues are not fully understood. Additionally, a soluble form of the leptin receptor exits (extracellular domain of the receptor) and serves as one of the protein species that binds leptin in blood.

In addition to the pivotal role leptin plays in the regulation of energy metabolism, leptin has a profound influence on the regulation of the neuroendocrine axis. Leptin regulates gonadotropin secretion and is believed to be a key signal linking energy balance, adiposity and induction of puberty in multiple species. Additionally, leptin has been shown to regulate growth hormone secretion from the pituitary and expression of key hypothalamic peptides involved in appetite regulation including neuropeptide Y.

Leptin and leptin receptor genes have been cloned in several livestock species. Recently, with the development of livestock species-specific reagents, the ability to characterize and quantify changes in leptin gene and protein expression under various physiological, pathological and nutritional conditions is possible. Data are emerging to implicate leptin as an important regulator of appetite, energy balance and reproduction in agricultural species.

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## Has Animal Science failed Society?

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Animal Science is, by convention, both applied and limited in scope. It is applied because it is directed towards the 'better' use of animals to serve man, usually in the form of a meal. Most Animal Science is limited because it focuses on what happens within the animal, a fascinating but rather inflexible link in the food chain, or detail on the broad environmental canvas. The success, failure (or complete irrelevance) of applied Animal Science is defined by what society understands as 'better' and by the extent to which animal scientists have contributed to this good.

Most people have a strong desire for meat, milk and cheese. For those too poor to enjoy the luxury of ethics, these things tend to be perceived as an unequivocal good, and this is biologically sound, since in these circumstances they are highly nutritious. When they become cheap, attractive and freely available, we overeat them, so risk both our health and our peace of mind. Fear and guilt redefine our concept of 'better' and redirect our concerns towards topics such as health foods, animal welfare and environmental protection. The Animal Production industry has undoubtedly succeeded in its aim of producing lots of good, cheap food. The direct contribution of animal science to this success will be examined critically; the evidence suggests that most of it would have happened anyway. However the industrialisation of animal production has created new problems for human health, animal health and welfare, and environmental pollution. It may be argued that the new role for animal science is to protect life from the perils of mindless productivity. It may be argued that it always was. To achieve this we must be wedded to science (and supported in our marriage), not the trophy mistresses of industry.

My main criticism of much animal science, as a science, is that it lacks panoramic vision. Scientists are constantly directed (by other scientists) to 'focus', an unnecessary exhortation since many have acquired tunnel vision by the completion of their PhDs. Science has two aims, discovery and understanding. Reductionist science is appropriate to the business of discovery; with each big new discovery exposing a thick album full of new stamps for collection. However I suggest that few of the big discoveries in biology have had much impact on animal production (antibiotics are a spectacular exception). Hormonal manipulation of animal production and reproduction was seen as right but repulsive; European society opting for the 'wrong but wromantic' notions of organic farming. If there is a role for Animal Science it should be directed towards the disinterested pursuit of understanding the role of farmed and wild animals in maintaining productive, sustainable ecosystems that best meet both the immediate needs of society and the long-term viability of the living environment. This will require more integrative science and less reductionism. Currently, I suggest, we are failing society in this regard. We are too interested in our own interests and most happy when talking to each other. Meantime, society flees from the stern discipline of scientific reason into the mushy embrace of organic farming, 'natural' foods and other manifestations of mindless fundamentalism. We may, at present, be failing society but we have never been more necessary.

# Helminth parasite problems and control options for the ruminant livestock industries

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Nematode parasite infections are now being recognised as possibly the greatest disease problem of grazing livestock industries, worldwide. Often parasites were considered to be of greatest concern in countries of the tropics and subtropics. This is based on the perception that parasites are either not as pathogenic, or are better controlled, in grazing livestock industries in the temperate regions of the world. Unfortunately this is not the case. The hidden costs of nematode parasite infection, particularly in young ruminants, are substantial even in Europe. This is now especially so, when one considers that maintaining livestock for longer periods on pasture is becoming and economic, environmental and animal welfare necessity.

The single greatest issue with regards to parasite control is the development of resistance to anthelmintic drugs. This has reached alarming proportions, particularly in the nematode parasites of small ruminants not only in countries of the humid tropics and subtropics, but also in countries with substantial sheep numbers in the more temperate regions of the Southern Hemisphere (eg. Australia, New Zealand, South Africa). Resistance to the whole range of modern broad spectrum anthelmintics is now a reality on sheep farms in many regions of these countries and inexorably increasing elsewhere, including Europe.

However, possibly the greatest threat to parasite control of grazing livestock in Europe comes with the rapid increase in organic farming practices. Countries within Europe lead the world in the move towards organically produced livestock products. This is fostered by the ever-increasing demands by consumers for food products free of any chemical residues and the perceived adverse consequences of using these chemicals on the environment. Legislation now proclaims that for organic farming, the prophylactic use of drugs (including of course anthelmintics) is prohibited. As a consequence, new and serious animal welfare issues are starting to emerge that are caused by distress suffered by animals due to uncontrolled parasite infections.

Thus two quite disparate issues, namely anthelmintic resistance and organic farming, have brought the matter of maintaining effective helminth parasite control to the forefront of grazing livestock management enterprises throughout the world. Clear messages are coming from the pharmaceutical industry that there is unlikely to be any completely new, alternative classes of anthelmintics reaching the marketplace in the near future. This is because of the high costs and risks associated with the animal health product research and development and the relatively poor returns on investment. Therefore it is critical to recognise that the available anthelmintics are very valuable resources and must be used sparingly and with sophistication. The only way to do this is to adopt the principals of Integrated Pest Management (IPM) for nematode parasite control in livestock. This requires the combination of a variety of non-chemical means of parasite control, together with very limited, strategic use of effective anthelmintics. This is the only way to maintain effective control for the forseeable future – which by definition, means sustainable parasite control.

# Immunonutrition: the nutritional control of acquired immunity to parasites

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**How do animal hosts control worms?** When an animal ingests an infective form of a gastrointestinal helminth (roundworm) from pasture, it can contain the infection by limiting the establishment, growth rate, fecundity and persistence of the parasite. This containment is achieved through the direct and indirect actions of the immune response. A helminth-specific immune response is, by and large, a local one and is achieved by an increase in the number of effector cells and the concentration of effector substances (such as specific immunoglobulins, proteases and mucin) in the gastrointestinal mucosa and lumen. The delivery of some of the effector substances is achieved through plasma leakage, part of which is irretrievably lost As these responses require nutrients for their expression and replenishment, it is not unreasonable to expect that host nutrition has the potential to affect the immune responses when nutrient resources are scarce. In this presentation we concentrate on the consequences of nutrition on the acquired immunity to parasites. Host nutrition can also affect innate immunity by, for example, making the gastrointestinal environment more hostile to parasites, but such effects will not be considered any further here.

**Nutritional control of immunity to helminths.** In ruminant hosts the phase during which the immune system starts to recognise the invading parasite (acquisition of immunity) could take time, up to several weeks in helminth parasitic infections. By expressing immunity, the hosts have the potential to eventually overcome current infection and limit further acquisition of infection. Host nutrition has thus the potential to affect the *rate of acquisition* and the *degree of expression* of immunity.

In previously naive hosts, such as lambs and calves around weaning, undernutrition with both macro- and micronutrients does not seem to affect how quickly hosts would acquire immunity. The time when the effect of the immune response on parasitic burden becomes apparent is independent of host nutrition, although it seems to be dependent on parasite species. This is despite the fact that the phase of acquisition of immunity is accompanied by a voluntary reduction in food intake (anorexia) and a consequent reduction in nutrient intake, which can last for a period of up to several weeks. The insensitivity of the rate of acquisition to changes in nutrient supply implies that this function takes priority over other body function in terms of nutrient allocation.

On the other hand, the degree of expression of immunity can be greatly affected by the nutrition of immune hosts, such as older lambs and ewes that have been previously exposed to parasites. Paradoxically, the function of expression of immunity appears to be penalised to a greater extent than other body functions, such as growth and reproduction, by undernutrition. For example, previously immune ewes suffer from a breakdown of immunity to helminths, at stages during which the demand for nutrients is high (ie late pregnancy and lactation) and supply of nutrients relatively low. This breakdown of immunity results in the re-establishment of a helminth population in the gastrointestinal tract. Reproductive performance, however, does not seem to be greatly affected by this breakdown of immunity. This implies that partitioning of a scarce nutrient resource is prioritised to reproductive rather than to immune functions. For an evolutionary explanation of the different sensitivities of the acquisition and expression of immunity to nutrition, see the framework of Coop and Kyriazakis (1999).

**Implications:** (i) Breakdown of immunity to helminths in already immune hosts is a major contributor to the epidemiology of the disease. Strategic nutrition can thus be used as additional means to control gastrointestinal parasitism within a herd or flock. The question then is which nutrients have the potential to affect the immune response. Current effort has been directed mainly towards investigating the effects of protein on the extent of expression of immunity (Houdijk *et al*., 2002). In principle, scarcity of any nutrient implicated in the immune response may cause some degree of relaxation of immunity.

(ii) Prioritisation of scarce nutrients towards productive functions rather than immunity, implies that expression of immunity in hosts that have high production output potential can be affected to a greater extent by scarcity of nutrients. This is because animals selected for high productivity are likely to direct more nutrients towards productive functions rather than towards immunity. The converse implication of this is that animals that have been selected for immunity to parasites would have lower production output. Currently these are suppositions and consequences of the above framework, but point towards an area of research where effort could be usefully directed: that of nutrition x genotype x immunity interaction.

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# Genetic control of host resistance to ruminant gastrointestinal parasites

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On a global scale, ruminant diseases caused by gastrointestinal parasite infection are the diseases with the greatest impact upon animal health and productivity (Perry *et al*., 2002). The problems associated with anthelmintic resistance amongst nematode parasites are well known, and considerable effort is now being made to devise alternative and complementary control strategies. Selection of animals with enhanced resistance is one such control strategy. This paper considers the genetic control of host resistance to gastrointestinal parasites and the selection of host animals with genetically enhanced resistance.

Convincing research has shown that many breeds of goats, sheep and cattle worldwide have better performance in the presence of worm challenge than other breeds available to the farmer (Gasbarre and Miller, 2000), i.e. they have enhanced resistance to, or tolerance of, infection. These include the Red Maasai, Barbados Blackbelly and Garole sheep and East African goats. In addition to the between-breed variation, there is also considerable genetic variation in resistance to nematodes within breeds of sheep and goats. This has been documented in Merino, Romney, Scottish Blackface and Texel Sheep, and Cashmere and Guadeloupe goats, amongst others.

Considerable research has quantified the genetic control of host resistance to nematode parasites in sheep under UK conditions. In Scottish Blackface lambs facing predominantly *Teladorsagia circumcincta* challenge, we have demonstrated that faecal egg count (FEC) (the primary indicator of relative resistance) is heritable, with this heritability rising from essentially zero in very young lambs to 0.33 at 6 months of age (Bishop *et al*., 1996; Stear and Bishop, 1997). Furthermore, in the same study we demonstrated that the 6-month FEC heritability is attributable to the ability of the host lamb to exercise genetic control over worm fecundity, rather than the total number of worms present in the gut. We have replicated the general findings of heritable variation in FEC in independent populations of Blackface and Texel sheep. These findings pertain to all eggs produced by worm populations characterised as strongyles. Another classification of nematodes commonly present are nematodirus. Although nematodirus egg counts are generally lower than strongyle egg counts, in both Blackface and Texel lambs nematodirus egg counts are almost always more heritable than strongyle egg counts. The genetic correlation between these two categories is close to 0.5.

Apart from growing lambs, the other category of sheep particularly susceptible to nematode infections is the peri-parturient ewe. We have found peri-parturient egg counts to be heritable ($h^2$=0.23), affected by the degree of metabolic stress the ewe is facing and positively genetically correlated with egg counts in the same animals as lambs (Bishop and Stear, 2001). The main importance of peri-parturient egg counts lies in consequences upon pasture contamination, however potentially they are a further indicator trait for use in selection programmes.

Knowledge of the immunological control of host response aids understanding of the host-parasite interaction and potentially supplies further indicator traits to be used in selection programmes. Increased parasite specific IgA activity in the abomasum is strongly associated with smaller adult female *T. circumcincta* (Stear *et al*. 1995). This relationship only holds with activity against fourth-stage larvae. In addition, there is a non-linear relationship between the amount of IgA in the abomasum and in the peripheral circulation (Stear *et al.* 1995). The number of worms in the abomasum is negatively associated with the transfer of antibodies to the circulation. Parasite specific peripheral IgA activity is highly heritable (Strain *et al*., 2002), but phenotypic and genetic correlations with adult female worm length and FEC have yet to be unambiguously determined. Other heritable indicators of immune response or the pathogenic impact of infection include peripheral eosinophilia, plasma pepsinogen concentration and plasma fructosamine concentration.

All the traits described so far require the animals to be challenged by nematodes. Genetic markers potentially allow selection of animals with enhanced resistance, irrespective of their challenge status. Markers that have been demonstrated as being predictive of resistance to *T. circumcincta* under Scottish conditions include the *DRB1* locus of the major histocompatibility complex and the interferon gamma locus (Coltman et al., 2001). Different alleles at these loci will confer different degrees of relative resistance. They are not markers for complete resistance.

Selection of animals for enhanced resistance will require incorporation of resistance into a broader breeding goal that will also include productivity traits. The genetic correlation between resistance and performance is required to achieve this. Consensus results suggest that the genetic correlation between FEC and growth rate is negative and moderate, approximately –0.20. Thus, as FEC decreases, growth rate tends to increase. The relative emphasis given to resistance to nematode parasites in the breeding goal depends upon the relative benefits obtained from having animals that are genetically more resistant. These benefits include (i) reduced anthelmintic requirements, hence a greater shelf life for currently used anthelmintics, (ii) improved animal health, welfare and productivity and (iii) decreased pasture larval contamination. This last benefit results in reduced larval challenge from pasture, hence greater productivity for all animals grazing the same pasture (Bishop and Stear, 1997 and 1999).

Commercial selection programmes for enhanced resistance are now underway in several countries, including New Zealand, Australia and the UK. In the UK, selection for resistance has formally begun in the Texel sire referencing

scheme, with the Suffolk sire referencing scheme following suite. Selection is currently based upon FEC measurements in growing lambs. Antibody responses and genetic markers are possible future additions.

In summary, the desire for sustainable production systems will be the major driving factor behind selection for nematode resistance in nematode livestock, worldwide. Additionally, within western countries, movements towards organic production systems will make selection for nematode resistance imperative, as treatment of infectedanimals will be constrainedin many circumstances. In general, it will be important to consider selection as being one tool within a raft of tools in an integrated parasite control strategy.

**Acknowledgements**
We wish to thank SEERAD, DEFRA, DFID, MLC and EU for funding, and Elite Texel Sires (UK) Ltd. for support and access to animals and data. Opinions expressed in this summary do not necessarily reflect those of the funding bodies.

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# Recent Advances in Foraging Theory for Herbivores

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**Introduction** My remarks explore the role of theory in making progress in science generally and particularly in ecology. I discuss what theory is (and isn't). I argue that theory is a fundamentally important part of doing science efficiently, and I discuss some exciting new approaches for combining theory, data, and statistics to enhance scientific understanding. I illustrate these approaches using examples from work on foraging by mammalian herbivores.

**What is theory?** The word theory is used in bewilderingly different ways. We often hear the phrase "while in *theory…"* juxtaposed with the more optimistically intoned "while in practice…." This not infrequently made comparison between the infirm and the substantial suggests that "theoretical" is synonymous with "irrelevant." Contrast this common use of the word with its broad meaning in science--a theory is a hypothesis that has withstood many attempts at disproof. For example, this is the meaning we have in mind when we speak of Darwin's *theory* of natural selection. Finally, theory is used to mean a mathematical statement that symbolizes processes in nature. In this context, theory is used to mean a mathematically precise expression of a research hypothesis and it is this definition of theory that I will use in my talk. I will use theory to mean "well composed models that make testable predictions about nature."

**Why bother with it?** Why is theory important? There are several reasons. First, it has been wisely said that "All models are wrong, but some are useful." Models are wrong because they are, by definition, abstractions--reductions in the detail of the natural world. Abstractions are ubiquitous in all creative work in science, literature, and art, because they force us to concentrate on the most *relevant* details in nature, the details that are judged by the scientist, the writer, or the artist to be the most important to the problem at hand. Without abstraction, science would be a complicated jumble of disconnected facts, but by using abstraction (read models or theory), we obtain a logical framework for connecting and organizing them. In addition, carefully composing our hypotheses as models allows us to combine the three fundamental routes to discovery—logic, mathematics, and scientific observation. Finally, good theory can be assembled in precise ways, allowing us to aggregate individual discoveries made by different people and at different times and places.

**How is theory tested?** The pejorative use of the word theory (In *theory…*) accurately reflects that theory alone is cheap—models become valuable only if they are tested against the observations of the states and processes they symbolize. How is theory tested? Development of foraging theory has suffered from a dismal history of testing models one a time. If we compare the predictions of a single model with observations and we find some semblance of agreement, we are tempted to proclaim the model "valid." But, testing a single model is like trying to push on a lever with no place to stand. A far more grounded approach is to assemble a set of models that make different predictions about the way the world works and evaluate the strength of evidence in data for these alternatives. Likelihood and information theory provide an accessible way to conduct this evaluation and so doing, provide a fundamentally important alterative to traditional hypothesis testing as a way of doing science. This alterative requires: 1) formulating a set of competing candidate models; 2) collecting data relevant to the predictions of the set; 3) using likelihood theory to estimate model parameters; and 4) using information theory to evaluate the information that is lost when a given model is used to approximate a complex truth. The model, or models, that suffer the least information loss provide the best approximations of reality, but they are "best" only relative to the models considered and in light of the data at hand. If the candidate set of models changes or the data improve, we may revise our evaluation. I review key concepts in these approaches, including the Kullback-Liebler distance, Akaike's Information Criterion (AIC), and measures of model selection uncertainty, in particular, Akaike weights.

**An example of testing foraging theory** We have been using these approaches in our laboratory to evaluate models of functional response for foraging herbivores. The functional response is a key concept in ecology, describing how the eating rate of animals responds to the availability of their food. We used likelihood-based techniques to compare four models of functional response of herbivores feeding in heterogeneous environments. These models represented the competing hypotheses that intake rate is controlled by: 1) plant biomass, 2) bite mass, 3) plant density, or 4) bite mass or plant density depending on a threshold. Predictions of models were compared to observations of the food intake rate of elk (*Cervus elaphus*), white-tailed deer (*Odocoileus virginianus)*, black-tailed prairie dogs (*Cynomys ludovicianus*), domestic rabbits (*Oryctolagus cuniculus*), and lemmings (*Discrostonyx groenlandicus*) feeding in patches where plant density and plant mass ranged over at least three orders of magnitude. The best approximating model ($w_r > 0.999$) portrayed a threshold ($d^*$, m) distinguishing mechanisms regulating functional response. When the distance between plants exceeded this threshold, intake rate responded to heterogeneity at the scale of the patch. When the distance between plants was less than the threshold, intake rate responded to heterogeneity at the scale of the leaf. We show that the threshold $d^*$ scales with body mass ($M$, kg) as $7.1M^{0.06}$. This relationship illustrates: 1) spatial pattern of plants at patch scales may influence intake rate only when plants occur at very low density; 2) herbivores of very different body mass respond to heterogeneity in a remarkably similar fashion; and 3) variation in plant characteristics at leaf scales influences the importance of heterogeneity at patch scales in regulating herbivore functional response.

**Conclusions** I close by recommending model selection as a fresh way to do science. It provides a strong framework for evaluating multiple competing hypotheses and, in so doing, offers strong inference about the operation of processes in nature.

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# Integrating livestock with the environment in upland systems

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**Introduction**. Livestock systems in hill and upland areas of the UK are characterised by extensive grazing, mainly by sheep and beef cattle. These areas also represent some of the most valued semi-natural habitats and landscapes in the country, with many areas being the subject to any number of a range of types of designation. This paper explores how, within upland systems, livestock can be integrated with their environment to achieve both agricultural and environmental objectives.

**Matching animal genotype to the environment.** Until the 1990s, as animal agriculture became more intensive, considerable emphasis was placed on selection of animals with superior levels of performance. In the cattle sector, for example, the importation of Continental breeds of cattle starting in the 1960s, led to a huge change in the breed composition of the UK beef cattle herd. The high levels of output from these genotypes was supported by modifying management to provide increased levels of nutrition by provision of highly digestibility forages and high levels of concentrate supplementary feeding. In the last decade increasing pressure on costs, a general reduction in inputs to livestock systems and a demand for more 'naturally produced' products has resulted in a need to develop systems that are more dependent on forages. Much anecdotal evidence suggests that some native breeds of sheep and cattle may be more suited to the utilisation of low quality roughages and in particular that their grazing behaviour may be more suited to the grazing of hill pastures. However, there is little scientific evidence that this is the case. One of the few examples is that of Wright *et al*. (2000), who demonstrated an interaction between genotype of cattle and nutritional environment. There was little difference in live-weight gain between yearling Welsh Black and Charolais-cross steers when they grazed permanent, grass/white clover pasture, but the live-weight gain of the Charolais-crosses was considerably lower than that of the Welsh Blacks on a *Molinia*-dominated semi-natural pasture. There is a need to identify whether such differences are more widespread between breeds of sheep and cattle and to what extent differences in grazing behaviour may be learned or genetic.

**Impact of grazing animals on biodiversity.** Manipulation of the three main factors, species of grazer, grazing pressure and seasonal pattern of grazing results in changes in sward structure and, in the longer term, can alter botanical composition. Differences in selectivity of grazing between species of livestock results in differences in diet composition, which in turn can alter sward structure and composition. For example, when grazing a *Nardus stricta*-dominated sward, the diet of cattle contains a higher proportion of *Nardus* than sheep, with the difference becoming greater as grazing pressure increases (Grant *et al*., 1985). Under cattle grazing, or mixed grazing by sheep and cattle, this leads to a reduction in the size and extent of *Nardus* tussocks. Changes in sward structure can have important consequences for the species composition and abundance of invertebrate species (Dennis *et al*., 1997), which in turn will influence the food supply for vertebrates such as bird and small mammals. Maximum diversity of plant species in grassland-based ecosystems tends to be achieved at a moderate level of grazing intensity (Grime, 1979). However, for many faunal species the structural heterogeneity of the sward is an important determinant of habitat quality. Milne (1996) therefore suggested that maximum biodiversity in grazed upland ecosystems, would, therefore be achieved if these areas comprised a patchwork of areas subjected to different grazing presssures, although there is currently a lack of information to test this hypothesis.

**Integrating livestock production with environmental objectives**. While much still has to be learned about how animals respond to their environment in terms of their foraging behaviour, especially at larger scales, and, therefore, how they affect the landscape, we have sufficient information to begin to devise systems that can achieve a balance of agricultural and environmental objectives. As with all multiple objective systems, trade-offs between objectives have to be balanced and compromises reached. This requires information, on the way in which the system functions and the consequences of management options, to be readily available and accessible to the decision makers. New decision support tools which encapsulate our current state of knowledge about these upland systems are becoming available and will be a valuable aid to decision making in the future.

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# How integrated livestock management can fit into the Rural Economy

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Language provides us with convenient generalisations that often conceal as much as they reveal. The term 'rural economy' fits into this category. In the UK the rural economy ranges from the lush pastures of Cheshire, interspersed with the residences of the wealthy, to the bleak Highlands and the isolated, windswept Western Isles. When we look for the optimum use of the resources involved in such areas no one solution can suit all. Equally our emotional attachment to rural economies often embraces the idea that they are timeless. In reality all rural economies are dynamic and some are changing very rapidly as this conference takes place. Probably the most useful task for those who seek to help farmers and planners is to come to an understanding of the moving economic equilibrium, towards which at any one time we are tending. That equilibrium can be represented as a balance between the inflows and outflows of people, money and physical resources at any one time.

This paper seeks to trace some of these factors as they are influenced by the application of integrated livestock management methods of upland farming.

**The crisis**

Upland livestock production continues to be the dominant form of land use in many rural economies. In most years it offers a very modest living to those who tend the animals and care for the countryside. More recently those incomes have fallen to such low levels that they do not generate a standard of living acceptable in 21st Century Britain. The position is intractable.

As real wages rise in the economy at large the expectations of those who live and work in the uplands, and especially their children, grow accordingly. Livestock production can only sustain such expectations if the individual farmer produces more. The options are not attractive. The land area of the farm may be increased so that more sheep or cattle can be produced. This provides severe challenges to the farmer in terms of both management and manual labour at key times of year. At the extreme it may lead to ranching systems with low standards of animal welfare, disease control and exposure to risks from marauding thieves. It also means that there will be fewer farms and fewer faming people in the local community.

Alternatively output may be increased on the existing area by higher stocking densities and the use of supplementary bought in feed. Again, there are some undesired impacts. First, higher density stocking may damage the natural environment. Second, reliance on bought in feed exposes the farmer market risks and will place him in competition with those in more favoured areas who can secure the feed at lower real costs.

In recent years this issue has become much more difficult as a result of an accumulation of new problems. BSE has damaged the image of the industry and the amount it can sell, cutting off vital export markets. Growing concerns about the environment and animal welfare have created a culture of suspicion about farmers and a readiness to regulate rather than to support. The Foot and Mouth outbreak has been unparalleled in its ferocity and has been most virulent in areas of hill farming.

Left to market forces alone there would still be animals in the uplands but many of the attributes of current farming systems would be lost. Perhaps most of all, there would be many fewer people working in the hills, villages would either lose population or have to find new sorts of economic activity to sustain their income. In the UK there seems to be a general consensus that this is not an acceptable outcome. As a result we have, within farm policy, for a long time provided additional help over and above what can be earned from the market to upland livestock farmers.

## The 'solutions'.

a) Realistically we need livestock in the uplands but by themselves they will not support the people who have to look after them'

   It will remain the core landscape use in many areas, determining the appearance of the countryside, the ecology it will support and the way in which human activity affects the wider environment, water, land and air, in these areas.

b) It can only survive on the basis of additional revenue flows these may come:-

   - From additional rural but non-farming activity
   - From the public purse in the form of direct payments – payments for public goods or social payments.

c) In both cases this depends on the consent of non-farming people

- The acceptance of industrial/tourist/service activity by planners and the local community – the changing nature of the population, changing skills requirement, exposure to new risks from decisions taken far away from the place of work.
- The willingness of the public to pay for public goods. Why should farmers be treated differently? What actual benefits do these payments secure compared with more spent on roads, houses, hospitals etc?

d) Consent depends upon shared understanding and agreed, acceptable goals.

- What the farmer has to offer, an approach that takes account of both the market pressures and the social importance in terms of environment, access and efficient resource use.
- Integrated livestock farming provides a framework for such a relationship.
  - It has to be explained
  - It has to move forward as new values and new technologies emerge
- Integrated livestock farming based on science and therefore grounded in research – not on ideology
- Integration has to extend beyond the farm into the market and into the community and its decision taking processes.

## Regulation of leptin expression in farm animals

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Leptin is a hormone mainly expressed by adipose tissue (AT) in mammals and/or by liver in birds, at levels that differ according to genetic, physiological, nutritional and environmental factors, as well as hormone treatments (Houseknecht *et al*., 1998; Barb *et al*., 2002; Chilliard *et al*., 2002; Taouis *et al.*, 2002).

AT leptin mRNA level was higher in sheep and goat subcutaneous than visceral tissues, and the opposite was observed in cattle; it was higher in fat than in lean selection line in sheep. Leptin gene expression was decreased by undernutrition and increased by refeeding in AT of cattle and sheep, and decreased during fasting but not after feed restriction in AT of pig. In the chicken, both AT and liver leptin mRNA were decreased by undernutrition. In lactating goats, the replacement of part of the dietary concentrates by soybeans did not change leptin expression. The injection of NPY in sheep, as well as growth hormone (GH) treatment of growing sheep and cattle increased AT leptin mRNA. In the pig, AT leptin mRNA decreased after GH administration and estrogen-induced leptin mRNA increased with age and adiposity. In the chicken, estrogen treatment decreased AT and liver leptin mRNA. *In vitro,* insulin and glucocorticoïds increased AT leptin mRNA in cattle and pig, and leptin production in sheep. However, these effects on leptin gene expression were inhibited by GH. In the chicken, liver but not AT leptin mRNA was either increased by insulin, glucocorticoïds and GH or decreased by glucagon treatments. Long daylength increased AT lipogenic activities and leptin mRNA, as well as plasma leptin in sheep. In sheep, AT leptin mRNA increased from prebreeding to mid-pregnancy and declined to prebreeding levels during early lactation (Ehrhardt et al., 2001).

Leptin protein was present in ruminant and sow milk and was expressed by the ovine, bovine and caprine mammary gland. In sheep, mammary leptin mRNA was high during early pregnancy and lower but still expressed during late pregnancy and lactation. Leptin was present in sheep mammary adipocytes, epithelial and myoepithelial cells during early pregnancy, late pregnancy and lactation, respectively (Bonnet *et al.*, 2002).

Plasma leptin in cattle and sheep was first studied thanks to a commercial “multi-species” kit. It was positively related to body fatness and energy balance or feeding level, and decreased by beta-agonist injection. The recent development of homologous RIA in sheep and chicken enabled more quantitative studies of changes in plasma leptin concentration. In the pig, plasma leptin pulse frequency was decreased by fasting and leptinemia was increased with age and adiposity. In ruminants, plasma leptin variations were explained for 35-50% by body fatness and for 15-20% by feeding level (Delavaud *et al*., 2000). The response of plasma leptin to meal intake was related positively to glycemia, and negatively to plasma 3-hydroxybutyrate (Delavaud *et al*., 2002). In sheep and cow, plasma leptin was high during late gestation, decreased around parturition and remained low during lactation (Block *et al*., 2001; Ehrhardt *et al*., 2001; abstracts of 2002 BSAS meeting).

The complex regulation of leptin expression and secretion, which is effected by tissues and animal species, is probably related to its diverse physiological and nutritional roles.

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## Welfare and Ethics

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The application of welfare science to the study of both domestic and wild animals raises important ethical questions which the science itself cannot answer, and my purpose in the paper is to give a framework for answering these questions. I shall also try to evaluate the contribution that welfare science can make to difficult questions of wildlife management.

Ethical questions are not settled merely by cost-benefit analysis, for reasons made apparent by the philosophical critique of utilitarianism. Ethical judgement requires some prior determination of which costs and benefits are relevant, and also a weighting of costs and benefits according to concepts of duty and desert. It also sets absolute limits beyond which utilitarian calculation may not proceed without the violation of a norm. The concepts of duty, right and desert are used to lay down and also to navigate these limits.

I argue that the non-human animals with which we normally deal, either in farming or in wildlife management, lack the mental capacities that would equip them for the moral life and therefore have neither rights nor duties. However we have duties to animals, and ethical questions must begin by specifying what these duties are. Duties arise from specific relations, and our duties towards domestic animals, companion animals and wild animals therefore differ according to the relations in which we stand to them.

Welfare science has developed along two paths: the physiological and the behavioural. The first path studies the physiological indicators of pain and stress, the second studies revealed preference and behavioural disorders. The first has its foundations in neuroscience and in Hans Selye¹s theory of the General Adaptation Syndrome; the second, exemplified in the work of Marion Dawkins, often borrows concepts from economics and related disciplines in order to pass from a study of behaviour to a conclusion about what is wanted or preferred. Both are attempts to assess Œpoor welfare¹ in terms of how it feels to the animal, without anthropomorphic analogies, and without unjustified assumptions about the nature or extent of an animal¹s cognitive powers. Both can be applied to ethical questions; but Œpoor welfare¹ will not in itself give us clear guidelines for the management either of wild or domestic animals, if we do not also consider the periods of good welfare against which it is set.

I go on to consider an argument that amalgamates the physiological and the behavioural, put forward by Patrick Bateson and Elizabeth Bradshaw in their studies of deer hunting. The authors argue, first on evolutionary grounds, that red deer are not adapted to the long chases to which hunting exposes them; secondly that this means that stress beyond what is acceptable is likely to be incurred; and thirdly that available measures of stress, of the kind standardly employed in the physiological approach, tend to confirm their conclusion. The argument is interesting in that it attempts to bring evolutionary, behavioural and physiological evidence to bear on a single question - which is whether the stress of a hunted deer has passed beyond Œacceptable¹ limits. Clearly the word Œacceptable¹ has moral implications, so the argument also shows the point at which a study of welfare might impact on ethical judgement.

I summarize the ripostes made to the scientific argument of Bateson and Bradshaw, and then go on to consider what the word Œacceptable¹ might mean in this and related contexts. The argument takes us back to the missing element in cost-benefit analysis - the element of the duty of care. What determines our duty of care towards an animal, and what costs may we impose on it, compatible with that duty? A study of our relations to domestic species shows that our thinking about duties is always and inevitably anthropocentric, and that our conception of duty is inevitably influenced by the human purposes which motivate us in dealing with other species.

In conclusion I survey two theories which attempt to answer the question of what is Œacceptable¹ without reference to the human purposes served: the theory that animals have rights (from which it follows that any violation of their rights is unacceptable) and the theory that stress which is so severe as to threaten homeostasis is unacceptable. The second theory is implicit in Bateson and Bradshaw, and provides an illuminating challenge to the moralist. It is difficult to apply, and open to criticism on the ground that it rules out too much; it also requires us to concentrate on only one factor in what may be a complex moral problem. On the other hand it promises to give an objective grounding to the duty of care involved in the treatment of wild animals, and, properly qualified, can suggest humane standards of husbandry.

# The legacy of positivism and the role of ethics in animal science

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A slow but steady shift in perspective on the need to address ethical issues within the professional activity of animal scientists has been underway for almost twenty years. Increasingly the issue is less *whether* animal scientists should be discussing ethical issues in their classes, at their professional meetings and in their interactions with client groups, but *how* they should do so. This paper will provide some arguments in support of this general trend, and will make some suggestions about how animal scientists can increase their capacity to address ethical issues as part of their professional responsibility.

Looking back on positivism from the vantage point of the 21st century, we can see that it was, in fact, at the vanguard of the philosophical movement we now call 'postmodernism'. The sciences of the 18th and 19th centuries were committed to foundational methods of inquiry. Some, following Descartes, stressed logic and mathematics as the foundational basis of natural laws, and argued that science must proceed through a rigorous process of deduction from basic definitions and self-evident principles of reason. Others, following Locke and the empiricists, took the foundational metaphor more literally, and argued that science could be built, brick by brick, from observations of the world. 'Positivism' is a word that came to be used to describe a number of alternatives to the foundational view. Some stressed the role of prediction, while others stressed verification. What they shared was the view that the quality of a scientific claim should not be assessed by looking backwards at its origins or foundations, but by looking ahead, to replication by others, to confirming or corroborating experimental results, or to real-world technological applications. In short, the focus shifted from foundations to justifications.

Several commentators have suggested that animal scientists reflect a positivist attitude toward their role, and that positivism has provided a rationale for excluding ethics from the professional life of the animal sciences. Positivism was a pervasive and very influential philosophy of science in the early 20th century, and its influence on the practice of science in both teaching and research settings was complex. On the one hand, the main doctrines of positivist philosophy dealt with methods of inquiry and the testing or justification of theory. This made it seem as if positivism itself had little to say about ethics or ethical issues. On the other hand, positivism *was* an ethic for the practice of science, and the rationale for being a positivist drew upon many principles that should continue to guide animal scientists in their approach to ethical issues.

During the early stages of the change from to foundations to justification, it was crucial to develop criteria that were independent from moral, ethical, religious or political norms. Some of the most notorious failures of early 20th century science—especially in genetics—involved putative justifications in light of contested moral norms. Nevertheless, there are three senses in which science—including animal science—cannot be made wholly independent from ethical norms. First, science is a process of rational inquiry and as such is ethically committed to a procedure of argumentation and consensus formation that presumes rules, common goals and a conception of fair play. Second, there are certain phenomena that can only be defined in light of ethical or normative reasons why they are of interest. Arguably, both health and welfare are such phenomena. Third, the rationale for undertaking many scientific investigations is often contingent upon the pursuit of personal or social goals, and these goals can themselves be subjected to ethical evaluation.

In each case, it is possible and important to distinguish the role of ethical justification from justification in terms of scientific criteria that appeal to logical consistency, predictive power, replication and the like. Much of the motivation for configuring the disciplinary norms of the animal sciences is based on the judgment that preserving and strengthening the distinction between ethical and scientific justification is an important thing to do. But this judgment is itself an *ethical* judgment, rather than a scientific one. Those who argued for positivism at its origins were not shy about basing their arguments on ethical grounds, but positivism was, in effect, undone by its own success. By investing so much intellectual energy into the protection of scientific criteria for the justification of theories and their results, animal scientists deprived themselves of the intellectual resources that they need to justify this very investment. And that is not to mention the ability to justify the use of their theories and results in the pursuit of both individual and social goals.

People who lack the ability to justify their activity in ethical terms often fall back on power plays of one sort or another. When scientists do this, they lose credibility and trust, for the value of science to society depends in large measure on the shared belief that appeal to coercive, political or economic power has no place in scientific justification. What is even worse is when the decline of critical, ethical argumentation actually allows scientists to align themselves with ethically indefensible practices and goals. Arguably, the decline of capacity and willingness to undertake ethical justification has reached crisis proportions in the agricultural sciences during the last four decades. As I indicated at the outset, I believe that the corner has been turned, and that we have now entered a phase of rebuilding that capacity.

As I wrote in my 1999 paper in the *Journal of Animal Science* (Thompson, 1999), the way to work out of this situation is, above all, simply to do it. Bioethics is emerging as an interdisciplinary field in which people with disciplinary training in the natural and social sciences collaborate with philosophers to examine critically production and

consumption practices, along with research, and to frame arguments about the ethical justifiability of these practices. These arguments will come to naught if they are not challenged and critiqued aggressively (but respectfully) by others. But if the argumentation and debate takes place *within* the professional world of animal science, it will build capacity and provide a basis for both wiser and more articulate defense and justification of practices among producers, consumers and citizens.

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# Ethical issues in animal biotechnology

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During the 20th century scientists have made genuine progress in explaining and modifying usefully the processes of life. The main leap forward was, of course, the rediscovery of Mendelian genetics at the beginning of the century. Since the 1930s this theory has been put to use in an ever more efficient and systematic way, particularly in breeding domestic animals with desired traits. Thus the animal breeder can now plan how he wants future generations of domestic animals to be, knowing that the plans will work.

A number of reproductive technologies have been developed to make selective breeding more efficient. Artificial insemination, freezing of semen, embryo transfer and trans-vaginal oocyte recovery followed by embryo production *in vitro*, are used increasingly to ensure that animals with good genetic potential produce more offspring than they would otherwise have had. These techniques reduce the generation interval, which means that the breeder's aims can be realised more rapidly. Looking ahead, sex selection is another reproductive technology which could prove useful in improving breeding efficiency.

But it is molecular genetics that holds the promise for a major leap forward in man's ability to control the processes of life. By means of so-called marker assisted selection it is possible to select highly specific traits at the genetic level. Moreover, through transgenesis, genes, and their phenotypic expression, may be moved across species barriers.

Until now molecular genetics has had relatively little impact on the breeding of domestic animals. This seems to have been for two reasons: First, scientific understanding of how the individual genes interact with the animal's phenotypic traits is at present limited. Secondly, the technology of gene transfer is still in its infancy.

Viewed in the context of these developments, the high profile unveiling of the cloned sheep "Dolly", by scientists at the Roslin Institute in Scotland in February 1997, was merely one further step in our efforts to interfere with the processes of life. Cloning somatic cells may turn out to be a useful way of disseminating the genes of female animals which possess desirable genetic potential; and the technique used to create Dolly may help in creating genetically modified animals from modified cells and thereby boost the development of transgenic animals.

However, viewed from another perspective Dolly made a big difference. This single sheep brought to many people's attention the fact that scientists had made a major breakthrough in their attempt manage and control life. It also gave rise to a widespread call for ethical limits to the interference with life to be established and enforced. Until recently the main limits to interference with life were of a technical kind: of what it is possible to do. Now, and increasingly, scientists are faced with ethical limits: of what it is *acceptable* to do.

The increase in power, and the potential increase of speed and efficiency that modern breeding and biotechnology presents, force us to recognise our moral responsibility and to discuss the limits of acceptability. In such discussion ethics provides a way of ensuring systematic and rational reflection on the moral issues involved within a framework of values and principles guiding behaviour.

The aim of this paper is to present an overview of various ethical considerations to which the applications of modern biotechnology in breeding of domestic animals gives rise. Furthermore, these considerations are to be subjected to critical reflection.

The first part of the paper will give an outline of how the use of biotechnology in animal production is perceived by the European public. The point of departure for this part will be the results from the Eurobarometer surveys on biotechnology, showing a European population sceptical towards the manipulation of animals. In the 1999 survey, the cloning of animals is grouped together with food biotechnology, as the least accepted applications of new biotechnology. This is so even though the assessed application of cloning is medical, a use that generally has higher public acceptance. On the basis of focus group interviews conducted by one of the authors, the findings of the surveys will be elaborated and explained in more detail.

The second part of the paper will provide a more systematic discussion of the considerations about animal biotechnology, partly drawing on the positions identified in the qualitative and quantitative analysis. Apart from well-known ethical categories and concerns like utility, risk, animal welfare, animal integrity, environmental concerns and human health, this will also include the fear that there is a "slippery slope" from the use of biotechnology on farm animals to uses on humans.

The final part of the paper will discuss possible regulatory reactions paying respect to the public scepticism, as expressed in the ethical categories identified earlier. Among other issues we will address the regulatory dilemma of constructing a consensus based regulation, when parts of the population are fundamentally opposed to genetically manipulated domestic animals.

# The ethical basis of intensive livestock production systems

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Many of the ethical issues regarding the livestock production systems of the late twentieth century have concentrated on the process of intensification. The extent of public disquiet at the welfare and ethical implications of intensification has been reflected in the burgeoning membership of animal welfare and animal rights organisations. The reasons for this increase as well as the impact that it has had on the livestock industry can be traced back to the 1960s, to factors such as the growing urbanisation of the population and especially the emergence of the animal rights movement which focused attention on a wide range of issues including the human exploitation of other animal species. This in turn led to a demarcation between those who supported the animal welfare cause and those who argued for animal rights.

The human understanding of the welfare needs of livestock has been greatly enhanced by developments in the fields of ethology and biochemistry. These developments have provided fresh insights into the experiences and perceptions of animals kept in intensive systems. The overall result of these developments is heightened public awareness of animal welfare issues, which has had an impact on both diet and consumerism. Thus there is evidence to indicate that some members of the public will only purchase animal products which they believe to have been produced in welfare-friendly systems. As a consequence of consumer pressure, food retailers have either initiated their own welfare assurance schemes or have linked with one or more of the schemes established from within the livestock industry.

The fact that the livestock industry has initiated assurance schemes illustrates the fact that many livestock producers have taken very seriously ethical concerns over intensive systems. Most livestock producers maintain that any proficient stockperson must be concerned for the well-being of their stock and that the care of animals must be their over-riding interest. Anything less would be deemed unethical and offensive. Producers often make such statements however, without reference to any ethical basis on which to substantiate their arguments in the face of growing criticism of their methods and systems of production.

This paper seeks to provide that ethical basis by arguing that:

1. The principle of respect for the essence of an animal is an acceptable ethical principle for the assessing of livestock production systems.
2. Respect is expressed agriculturally in stewardship and stockmanship, which consequently become the criteria for judging the ethical acceptability of livestock systems.

The implications of applying the respect principle to various methods of livestock production are considered, including the battery production of eggs, the use of farrowing crates for pigs and routine animal mutilations. It is argued that some techniques and procedures employed in intensive systems of livestock production, such as farrowing crates, are ethically acceptable, whilst others, such as current battery production systems for poultry, are not.

Factors likely to encourage or hinder acceptance of the respect principle are also identified and examined. Of these, the likelihood of increased costs for both producer and consumer is identified as one of the most negative. Factors likely to encourage the wider acceptance and adoption of the respect principle include the development of consumer/retailer/producer partnerships through Farm Assurance Schemes, an increased commitment in the livestock industry to training in stockmanship skills and greater political commitment, not only to animal welfare legislation, but to the viability of those producers affected by it.

It is concluded that the application of the respect principle must therefore result in some major modifications to certain production methods, which will lead in turn to significant improvements in the welfare of the animals concerned. It is also recognised that intensive livestock production is not by definition unethical and that there are certain practices and systems within this category which are perfectly acceptable and which actually improve the welfare of the animals concerned.

# The 'Livestock Revolution' – can poor livestock-keepers benefit?

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**Abstract**
The projected increase in global demand for animal products, recently termed 'the Livestock Revolution'**,** presents livestock keepers, in both the developed and the developing world, with many opportunities but also some problems. The increase in demand, mainly in South and South-East Asia, is described, disaggregated by region and product type. Some of the implications, in terms of the environment, public health, grain prices etc, of this increase in demand are also identified.

A major concern is raised that the household-level demand, indeed desperate need for an increase in the level of animal product consumption, is often not explicitly considered in macro-economic models. The chronic protein and micro-nutrient malnutrition of adults and children from households raising livestock is widespread in the developing world. Some the opportunities for this group of people, too poor to eat their own animal products, to improve their livestock production, increase incomes and thereby increase their consumption of animal products are identified.

Finally, the research and development needs to meet this rising global demand and ensure that poor livestock producers benefit are outlined.

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# Are enzymes useful in ruminant diets?

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**Introduction** Cellulose and hemicellulose are the major structural carbohydrates present in forages and form between 30 and 60% of the forage component of ruminant diets. The complex network of structural carbohydrates and lignin generally leads to low digestibility and limits the efficient utilisation of forages by ruminants. This situation occurs in both developed and developing countries, and in the latter it is particularly pronounced as much of the forage component is based around the use of crop residues (Owen and Jayasuria, 1989). Because forage costs are significantly lower than those of other dietary ingredients, improving forage quality has been a major objective for many research programmes in both the developed and developing world. Improvements in forage quality have been sort through a number of different strategies. These have included conventional breeding techniques, which have included the integration of mutant genes, leading to the development of Brown Midrib varieties of maize and the use of chemical and biological additives. Enzyme supplements are commonly used to improve the nutritive value of feeds for non ruminants and as silage additives where they have been shown to improve silage fermentation, feed intake and performance. Recent work with ruminants has however focused on the use of enzyme supplements to improve feed efficiency by the use of "direct-fed" fibrolytic enzymes. This strategy involves the application of enzymes to feed at or only hours before feeding. These studies have yielded very variable production responses. For any new technology to be implemented widely, the responses achieved must provide an acceptable level of consistency and predictability. The current paper reviews developments in enzymology, production responses achieved and the effects on nutrient digestion.

**Enzyme supplements** During the last four decades, significant progress has been made on the biochemistry and enzymology of cellulases and hemicellulases (Bhat and Bhat, 1997; Bhat and Hazlewood 2001). A wide variety of bacteria and fungi, aerobes and anaerobes, mesophiles, thermophiles and psychrophiles, capable of producing high levels of cellulases and hemicellulases have been isolated and their enzymes purified and characterised. In addition, qualitative (dye release or zone clearing) and quantitative (reducing sugar determination) assay methods were developed using synthetic and model substrates. Generally, most cellulase and hemicellulase components are optimally active between pH 3.5 – 6.0, but the optimal temperature for activity varies depending on their source (psychrophilic enzymes, 30–45$^{o}$C; mesophilic enzymes, 40–60$^{o}$C and thermophilic enzymes, 60-75$^{o}$C). Based on substrate specificity and mode of action studies, cellulases have been grouped as endoglucanases, exoglucanases and β-glucosidases, while the hemicellulases include mainly xylanases and xylan de-branching enzymes. In fact, many fungi produce these enzymes as separate entities, while some anaerobic bacteria (*Clostridium thermocellulum* and *Clostridium cellulolyticum*) and anaerobic fungi (strains of genera *Neocallimastix, Piromyces* and *Orpinomyces*) produce multi-enzyme complexes having both cellulase and hemicellulase activities. Recently, many cellulase and hemicellulase components have been crystallised, their 3-D structure determined and classified based on sequence similarities into glycoside hydrolase. Interestingly, the members of the same family possess similar fold, 3-D structure and follow the same catalytic mechanism. Furthermore, it has been well established that many cellulase and hemicellulase components are bi-modular proteins with a large catalytic and a small binding modules.

**Production responses** With few exceptions (Lewis *et al.,* 1999) the majority of authors have reported that the application of direct fed enzymes produced no significant effect on feed intake. However, many workers have drawn attention to the fact that small numerical increases occurred (Yang *et al.,* 1999; Lewis *et al.,* 1999; Rode *et al.*, 1999; Kung *et al.,* 1999; Schingoethe *et al.,* 1999; Phipps *et al.,* 2000). While significant increases in actual milk production have been reported by Kung *et al.,* (2000), Yang *et al.,* (1999, 2000) and Lewis *et al.,* (1999), others workers have only noted numerical but non significant increases (Schingoethe *et al.,* 1999; Beauchemin *et al.*, 1999; Rode *et al.*, 1999; Phipps *et al.,* 2000). In addition many of the authors have also expressed results in terms of fat corrected milk (FCM) yield. In some cases, where enzyme application decreased milk fat content, a significant response in actual milk yield became non-significant in terms of FCM yield (Kung *et al.,* 2000; Rode, *et al.*, 1999; Yang *et al.,* 2000) and in others, when milk fat content increased non-significant responses in actual milk yield became highly significant responses in FCM yield (Schingoethe *et al.,* 1999). In the studies reviewed the range in actual milk production response varied from 0 to 6.3 kg/d. Changes in milk composition due to the application of direct fed enzymes are variable. While Schingoethe *et al.* (1999) noted significant increases in both milk fat and protein content, marked reductions have been reported in some studies by Kung *et al.* (2000) and Phipps *et al.* (2000) while yet others (Yang *et al.* 1999) have recorded no effect on milk composition.

**Digestive processes** It is reasonable to assume that the primary response to expect from the use of fibrolytic enzymes is an improvement in *in vivo* fibre digestibility. Although enhanced *in sacco* digestibility has been demonstrated, reported responses in dairy cows have been very variable. In some experiments total tract digestibility of fibre has been improved significantly (Yang *et al.,* 1999; Beauchemin *et al.,* 1999) or at least numerically (Yang *et al.,* 2000) but in others there has been no response (Sutton *et al.,* 2001). Two trials have been reported using cows with duodenal cannulas (Beauchemin *et al.,* 1999; Sutton *et al.*, 2001). In both, a fibrolytic enzyme was applied to a TMR and fibre digestion was reduced in the rumen but enhanced in the post-ruminal tract suggesting that the site of action of the enzyme was in the intestines. However, no such response was seen when the enzyme was applied to the concentrate only or was infused into the rumen, which further confuses the interpretation. One possible reason for the inconsistent

digestion responses is that, in several trials, enzyme treatment has been associated with increased rate of particle outflow from the rumen possibly caused by lower rumen fluid viscosity (Beauchemin *et al.* 1999; Yang *et al.*, 1999; Sutton *et al.*, 2001). If retention time is reduced then the potential benefits of any enhanced fibrolytic activity may be lost.

**Conclusions** On a global basis the importance of forage in ruminant diets is such that improving its quality must remain a major objective for research programmes in both the developed and developing world. Studies reviewed in the current paper have shown that although the application of direct fed enzymes increased nutrient digestion in almost all cases the increases were generally small indicating only modest efficacy of those enzymes. While source of enzyme, method and rate of application and stage of lactation have all been examined as possible factors which might influence production responses the results have failed to show a consistent pattern. Despite intensive studies on biochemical properties, substrate specificity, mode of action and structure/function relationships of cellulase and hemicellulase components, the combination of enzyme components and their properties required for improving forage utilisation by ruminants is still unclear. However, efforts should be focussed on the selection of enzyme components that are shown to be highly active and stable under the prevailing pH and temperature rumen conditions. The importance of this statement is emphasised by recent calculations which have shown that in the case of the enzymes used at Reading 0.66 of their enzyme activity was lost at pH 6 and at a temperature of $38^0$C, which reflects reasonable rumen conditions. Under these conditions the added enzymes would contribute little to rumen fibrolytic activity and it is postulated that the modest and variable production responses noted in many of the papers reviewed is due to inappropriate selection and lack of characterisation of the enzyme additives.

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# Feed enzymes for ruminants. The need for a rational screening system

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Enzymes that degrade the plant cell wall, collectively termed cellulases and hemicellulases, have attracted considerable research efforts recently, because of their potential to be used as additives for animal feeds. Widely used in monogastric animals, mainly to remove antinutritional factors (Bedford, 2000), enzymes are increasingly used in ruminant diets to enhance feedstuff degradability, following results from feeding trials where positive responses in rumen digestion and animal production were observed (Beauchemin *et al.*, 1995; Feng *et al.*, 1996; Yang *et al.*, 1999). Enzymes could also provide an environmentally friendly alternative to the use of antibiotics as feed additives for ruminants.

However, the nutritional response of ruminants to supplementation with enzymes has been mixed, and appears to depend on many factors. The high complexity of the rumen ecosystem makes it difficult to define which, if any, enzyme activity is necessary to elicit the responses. The biochemical characteristics of the enzyme preparations may dictate the nature of the effects, but are often overlooked or poorly defined prior to use. In addition, because of the impossibility of testing all enzymes *in vivo*, there is the need for the development of a suitable *in vitro* screening system to allow the most promising enzyme mixtures to be identified, and then used under *in vivo* conditions.

Enzymes currently used in animal nutrition are often extracts from fungal cultures (mainly from *Aspergillus sp.* and *Trichoderma sp.*), containing mixtures of different enzymic activities. The array and magnitude of these activities are influenced by the microorganisms used for their production (genus, species and strain), and the conditions used for growth (substrate, pH, temperature, etc.). Small changes in the way enzymes are produced, even from a single organism, may affect the responses in ruminants. Nsereko *et al.* (unpublished observations) examined the effect of carbon source on the production of fibrolytic enzymes by *T. reesei* and found large variations in the main and side enzymic activities. Xylanase activity was highest when *T. reesei* was grown in wheat straw or maize silage, compared to alfalfa hay, xylan, TMR, timothy hay or microcrystalline cellulose. In addition, the cellulolytic system was optimized using alfalfa hay, maize silage or timothy hay as substrates.

The determination of the enzymic activities present in the preparations is based on the release of hydrolysis products from model substrates, under optimal and standardized conditions (e.g. Wood and Bhat, 1988). However, these optimal conditions for the enzymes are not similar to the *in vivo* situation (Sabatier and Fish, 1996). In order to obtain more realistic estimates, it is imperative that enzyme assays be conducted under conditions that closely resemble those found in the feed or in the animal, depending upon the site of enzyme action. Such an approach has been recently undertaken by some groups (Colombatto *et al*., 2000a; Morgavi *et al*., 2000; Wallace *et al*., 2001). Furthermore, Colombatto *et al.* (this meeting, a) characterized a group of 23 commercial enzyme samples and developed multiple regressions between the enzyme activities on model substrates and the responses on *in vitro* rumen DM degradability of alfalfa and maize silage. However, given the complexity of the plant cell wall and the rumen ecosystem in itself, it is unlikely that this kind of analyses would accurately predict the responses under all situations.

The recognition of the above problem has led researchers to search for alternative *in vitro* rumen bioassays to complement the biochemical characterization. Although the animal will always be the ultimate test for a given additive, *in vitro* tests are convenient as first screening steps, especially when large numbers of samples are to be evaluated.

Of the *in vitro* techniques available, one of the most popular is the Tilley & Terry (1963) *in vitro* digestibility system. Although generally well correlated with *in vivo* data, it is essentially an end-point technique, and provides no information regarding the dynamics of fermentation. As one of the most documented responses of enzyme additions to feeds has been the increase in the rate, but not extent, of feed fermentation (Yang *et al.*, 1999; Colombatto *et al.*, 2000b), the use of this technique may not be appropriate. In contrast, *in situ* techniques (e.g. Ørskov *et al*., 1980), which are based on artificial fiber bags placed in the rumen, provide information about dynamics of feed disappearance in the rumen. However, these techniques are less attractive due to high associated costs and the limited number of samples that can be analyzed at one time. A viable alternative to the *in situ* technique is offered by the filter bag technique in revolving incubators (Daisy II, ANKOM Corp., NY, USA). One of the main advantages of this technique is that it allows determinations of DM, NDF and ADF to be carried out using the same samples, thereby minimizing the associated errors. This technique has been used successfully to determine the effects of direct infusion of enzymes (i.e. no pre-treatment period) into fermentation flasks (Colombatto *et al*., 2000c).

Finally, gas production-based techniques offer an advantage over gravimetric methods because they account for both soluble and insoluble substrates (Pell and Schofield, 1993). Several systems with varying degrees of complexity have been proposed (Menke *et al*., 1979; Theodorou *et al*., 1994; Cone *et al*., 1996). Recently, the semi-automation of an existing system (RPT, Reading Pressure Technique) has allowed a large number of samples (up to 375 individual fermentation flasks) to be analyzed at one time (Mauricio *et al*., 1999). This technique was developed to examine simultaneously the rate and extent of both gas release and substrate degradation. By using this approach, it becomes possible not only to fractionate the feed into its main carbohydrate components, but also to provide an estimate of their extent of degradation (Beever and Mould, 2000). The RPT technique has been used to identify even slight changes (i.e. 15 mg/g OM degradation) in fermentation activity due to enzyme addition to feeds (Mould *et al*., 1999; Colombatto *et al*., 2001; Colombatto *et al*., this meeting, b).

In conclusion, rational selection of feed enzymes for ruminants should include a complete biochemical characterization of the candidates, performed under physiological conditions. Then, the candidates should be examined in presence of rumen fluid in order to test their stability and ability to influence the fermentation process. Several *in vitro* techniques are available, of which the ANKOM system and a high throughput method combining gas production and feed degradation such as the RPT technique appear as the most promising.

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# Enzymes in feed resources for farm livestock – overview

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**Introduction** Within 15 years after introduction, the application of enzymes in the feed industry has gained general acceptance. Even though the range of feed enzymes has not really been extended in this period, a lot of progress has been made, particularly in a better understanding of the mode of action of these enzymes. The main enzymes used are those able to hydrolyse non-starch polysaccharides (NSP) and phytase.

**NSP-degrading enzymes** The use of NSP-degrading enzymes as feed supplements has consolidated around their use in wheat- and barley-based diets for broilers and, to a lesser extent, piglets. The continuously growing understanding of their mode of action, and in particular the interaction with the animal's digestive and gut microbial system, affects the way feed enzymes are applied. Utilisation of these enzymes in diet formulation alleviates raw material constraints and increases nutritional value of a specific diet. Depending on economic circumstances, and dietary constraints, NSP-degrading enzymes can save more than 2.- ?/t of broiler feed. But they also fit in the context of taking measures to improve animal health and welfare as well as for reducing environmental pollution.

**Phytase** About two-thirds of the phosphorus in vegetable raw materials used in animal feeds is present in the form of phytate, which is poorly available to monogastric animals. Addition of microbial phytase has long been known to improve phosphorus digestibility (Nelson *et al*., 1968). A viable commercialisation of this concept finally started in 1991, with the introduction of Natuphos®. Since then, hundreds of scientific papers have dealt with this feed enzyme, investigating its mode of action and efficacy in different species of monogastric animals, including pigs, broilers and laying hens. International independent research established an equivalency of 500 FTU/kg diet to 1.0 g P from monocalcium phosphate (MCP) in monogastric animals (for laying hens 300 FTU). Further research has shown that phytase not only increases the digestibility of P, but that of other minerals and amino acids too. In poultry, an additional effect of Natuphos® on dietary metabolizable energy has been shown. Using a meta-analysis of research results, Kies *et al*. (2001) calculated the additional effects of phytase on amino acids and energy utilisation. Based on their findings, the feed industry is able to save up to 4.- ?/t of feed for broilers, which can be (much) higher when the P-level of the diet is maximised.

**Practical application issues** Enzymes are bioactive proteins and as such vulnerable to de-activation by individual or combined effect of time, temperature and humidity, like during storage or feed processing. The problem of this "thermo-instability" can be overcome by different means: overdosing of the enzyme, by a protective enzyme-product formulation, or by using intrinsically more thermostable enzymes. In most practical feed production processes, a relatively simple formulation will be sufficient. Most feeds, like those for grower/finisher pigs, sows and laying hens, are pelleted at a temperature below 80°C or are not pelleted at all. For most broiler feed, a formulation like that of Natuphos® G is very good. Withtemperatures exceed 85°C large enzyme activity losses may occur, but these can be overcome by special product formulations, e.g. application of a coating. These are, however, not without risk: bioavailability of the enzyme can significantly be reduced (Klein Holkenborg and Braun, 2001; Kwakkel *et al*., 2002). The authors showed that a coating of phytase granules in general improved pelleting stability, but the enzyme effect on animal performance declined with the intensity of coating.

**New developments** Developments that can be envisaged at short term are enzyme products that are more (thermo-) resistant at present-day feed processing, either by searching for or creation of thermostable enzymes, or by development of more efficient product formulations. In the latter case, it may be hoped that the ultimate objective, activity within the animal's gastro intestinal tract, receives more attention. Although a large number of enzyme products appeared on the market in the last ten years, no new concepts or new enzyme activities were among them. It may be expected that such new concepts or enzyme activities appear in the next few years, fuelled by the search for effective enzymes for maize-soya diets and as alternative for antimicrobial growth promoters.

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# Zoonotic risk assessment and risk management in the red meat sector

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Zoonoses are those diseases of animals that can be transmitted to man or vice versa. These may be caused by bacteria, parasites, or more rarely by viruses. This paper will concentrate mainly on the zoonotic bacteria *Salmonella* spp., *Campylobacter* spp., *E. coli* O157 and *Yersinia* spp. Foodborne disease, often described as food poisoning, is defined as disease due to the consumption of food contaminated with microorganisms or their toxins. In 2000, the most common causes of food poisoning in humans in Great Britain were *Campylobacter* spp. (60340 cases) and *Salmonella* spp. (16567 cases). Less than 100 cases of *Yersinia* spp. infections were reported in humans in England and Wales. Results from surveillance of zoonotic organisms in farm livestock were presented at two conferences organised by MAFF in 2000. The main results are outlined in table 1. It is not possible to guarantee the complete absence from meat of bacteria that can cause infections in people. Thorough cooking of foods should kill all vegetative bacteria. Nevertheless, it is also important that steps are taken throughout the supply chain to minimise the transfer of the bacteria that could cause foodborne disease.

**Table 1** Percentage of faecal samples from which zoonotic organisms were isolated (Dalziel, 2000; Davies, 2000; Evans, 2000; Newell, 2000; Paiba, 2000)

| | Cattle | Sheep | Pigs |
|---|---|---|---|
| *Salmonella* spp | 0.2 | 0.1 | 23.0 |
| *Salmonella* Typhimurium | 0.2 | 0.1 | 11.1 |
| *Campylobacter* spp (all) | 24.5 | 17.0 | 94.5 |
| *Campylobacter jejuni* | 11.2 | 11.3 | 3.4 |
| *C. jejuni; C. coli; C. lari* | 13.5 | 15.8 | 87.1 |
| *Yersinia enterocolitica* | 6.6 | 13.7 | 26.1 |
| *E. coli* O157 | 5.4 | 2.0 | 1.2 |
| VTEC O157 | 4.7 | 1.7 | 0.3 |

***Salmonella*** *Salmonella* is more common in pigs than cattle or sheep. *Salmonella* is widespread and may be introduced to livestock premises by a variety of routes. Once introduced, the infection tends to be recycled through the herd or group of animals. The level can be maintained at a higher level by poor management and poor hygiene. The BPEX/MLC Zoonosis Action Plan (ZAP) is an initiative to reduce *Salmonella* on GB pig farms. This will identify farms where there is a high prevalence of *Salmonella*. Producers from these farms will be provided with specific on-farm advice as to how to reduce the levels of *Salmonella*. Producers will also be required to develop, in conjunction with their farm veterinary surgeon, an action plan to reduce the incidence of *Salmonella* on their unit. ZAP will become an integrated element of farm assurance schemes and provide a further tool to reduce the threat of *Salmonella* in pigmeat products.

***Campylobacter*** Transmission of *Campylobacter* infection has been associated with raw or undercooked meat (especially poultry), unpasteurised milk, bird-pecked milk on doorsteps, untreated water, and domestic pets with diarrhoea. Person to person transmission can also occur if personal hygiene is poor. Most human infections are caused by *Campylobacter jejuni*. Most pigs carry *Campylobacter* spp. in the gut but in the majority of cases the species present is not *C. jejuni*. Pork is assumed to constitute only a minor source of human infection. The levels of *Campylobacter jejuni* carried in the gut of animals at slaughter are higher in cattle and sheep than in pigs.

***Yersinia*** *Yersinia* spp. are found in the gastrointestinal tract of many species of wild and domestic animals and birds, where infection is usually asymptomatic. People can pick up the infection from contaminated food and water (organisms can multiply in food at 4°C) or through direct contact with infected animals. Person to person spread may occur. *Yersinia* spp are very common in pigs in many countries. Improved hygiene at slaughter to reduce the faecal contamination of the carcass is important if contamination of the finished product is to be minimised. The effect of the control measures suggested for *Salmonella* on the incidence of infection in pig herds should be monitored.

***Escherichia coli* O157-H7** *E. coli* O157 is transmitted by food and water, directly from one person to another, and occasionally through occupational exposure. As few as 10 - 100 bacteria can cause symptoms in humans. Healthy cattle and sheep can be excretors of *E. coli* O157. The organism can persist in manure, water troughs, and other places on farms. Although most human infections occur in urban areas, people living in rural areas may be at greater risk of infection, presumably because of greater exposure to livestock. Some foodborne outbreaks have been traced to foods derived from cattle, especially ground beef and raw milk. Meat probably becomes contaminated at the time of slaughter; mincing may compound the problem by introducing the pathogen into the interior of the meat, where it is more likely to survive cooking.

The recommendations in the Pennington Report (The Pennington Group, 1997) applied to conditions on the farm and throughout the food chain and included:

- scrupulous personal hygiene on the farm

- presentation of animals in an appropriate clean condition for slaughter
- the promotion of good hygiene practice in slaughterhouses
- the use of the hazard analysis and critical control point (HACCP) system in the slaughterhouse and in food businesses
- separation in storage, production, sale and display between raw meat and unwrapped cooked meat/meat products
- food hygiene should feature, where possible, in the primary and secondary school curriculum
- food hygiene should be taught widely to caterers and food handlers.

*E.coli* O157-H7 is sensitive to heat and the organism is killed by proper cooking of meat products (core temperature of 70$^{o}$C for at least 2 minutes). Procedures to deal with this organism need to be applied across the whole of the livestock and meat supply chain. MLC has been proactive in facilitating initiatives introduced in response to the Pennington report including the MHS Clean livestock policy; the introduction of HACCP in abattoirs and processing plants and the introduction of HACCP in butcher's shops.

**Action to manage risk from zoonoses** Key approaches to reducing the risk of foodborne disease include:
- Reduction of zoonotic pathogens in food animals
- Prevention of cross contamination during activities such as transport and slaughter
- Safe processing of foods underpinned by HACCP (Hazard Analysis Critical Control Point)
- Safe handling and preparation of food in the catering sector, including the use of HACCP and effective enforcement
- Safe handling and preparation of food in the home
- Enhanced surveillance of foodborne disease, to monitor effectiveness of controls

A number of control measures have been introduced to reduce the likelihood of contaminated food entering the food chain and to prevent spread by cross contamination. There is a general requirement for food businesses to identify and control the risks associated with their products. HACCP is internationally accepted as the most effective way to manage food safety and protect public health and is being promoted throughout the abattoir and processing sector.

Knowledge about the epidemiology and surveillance of zoonotic diseases is essential for the design, implementation and evaluation of control programmes. The objectives of zoonoses surveillance and control can only be accomplished with reasonably complete and reliable data. Further research is required in this area. MLC has commissioned a review of *Salmonella* research with a view to setting future research funding priorities. MLC is funding research on risk assessment and evaluation of critical control points for pig production and a study of on-farm *Salmonella* monitoring and control.

The industry has demonstrated that it is proactive and has incorporated the DEFRA Code of Practice for the Prevention and Control of *Salmonella* on Pig Farms into the farm assurance schemes.

**Transmissible spongiform encephalopathies** No discussion of risk in the red meat industry is complete without a few words on TSEs. Managing the issue of BSE in cattle depended on understanding the science behind the issue and putting controls in place to protect consumer health. Meaningful communication of the issues to consumers depended on an understanding of consumer concerns. The potential risk that BSE has entered the sheep flock is recognised. The National Scrapie Plan, to select sheep for resistance to scrapie, is a key element in protecting consumers against this potential risk.

**Conclusion** Action is required through the food supply chain to reduce the risk posed by zoonotic organisms. It is important that responsibility for food safety is taken by participants in every sector of the industry.

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# Market perceptions of food and feed safety

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**Introduction** The last decade has witnessed a dramatic increase in the introduction of risk management and safety assurance throughout the UK's food and feed processing and manufacturing industries. This has resulted in part from changes in legislation, but more significantly it has reflected a fundamental shift in the market environment in which the food-chain industries operate. Risk management was established as a key requirement when the emergence of food safety problems in the 1980s became a matter of public concern. Retailers responded by driving the safety assurance 'down' the food-supply chain, and politicians by moving food-chain regulation up their list of legislative priorities.

Over the ensuing period systems of risk management in industry have developed almost beyond recognition, but there has been less progress in addressing, or even properly understanding, the forces influencing market perceptions of food and feed safety.

**What is safety?** The Oxford Dictionary defines *safety* as *freedom from danger or risk* - conveying an impression of an absolute state. In reality safety is always a relative term, since risk can only be reduced to a level which the more pragmatic mathematicians might describe as zero 'for all practical purposes'. More importantly perhaps, risk can only be quantified on the basis of human knowledge and experience; we can never be absolutely certain about the unknown. Thus those who demand 'absolute safety' or 'absolute evidence of safety' are asking for something that is impossible to deliver.

Risk management can provide a robust scientific framework for minimising risk and maximising safety. Howevr, market perceptions of safety are influenced by a range of factors that are not risk-related. It is therefore misguided to believe that market perceptions of food and feed safety can be addressed simply by better risk management in industry.

**Definitions and terminology** Study of risk, conventionally described as risk analysis, is regarded to consist of three main components: risk assessment; risk management; and risk communication. Risk assessment involves the identification of potential hazards, their characterisation, quantification and evaluation as risks. Risk management is the process whereby risks may be controlled to reduce them to acceptable levels, for example through codes of practice, quality management or quality control systems or some type of Hazard Analysis Critical Control Point (HACCP) system. Risk communication is the process whereby information about risks is communicated to those who need to know, or wish to know, what the risks are and how they are being controlled. Risk assessment and risk management are crucial in the technological challenge of identifying and controlling risks – they are areas of risk analysis in which the food and feed industries have become increasingly proficient. On the other hand market perceptions of safety are determined largely by risk communication, and here industry and government has frequently had limited success.

**Risk communication** From a manufacturer's standpoint, the challenges of achieving successful risk communication and a high market perception of food or feed safety are very considerable. Firstly, the communication process must be multi-directional, recognising that suppliers, regulatory and enforcement agencies, farmer customers, food retailers, consumers, non-governmental organisations (NGOs), campaign groups and the media are all 'stakeholders' to which communication must be addressed. Secondly, the communication must be multi-form, recognising that its detail and approach must be targeted to the information needs of the various groups, and make use of the most effective means of communication. Finally, the communication must be part of an overall communication policy and strategy. The communications arena has few boundaries, and it is easy for a particular communication message to be 'drowned-out' or overtaken by messages issued by other 'stakeholders' who may be seeking to advance their own particular agenda or point of view. Faced with this challenge it is not surprising that most manufacturers adopt a low profile in general public debates on risk. They mainly focus their attentions on business-to-business communication and, communicating with consumers, on non-statutory product labelling, product-assurance leaflets and advertising.

**Market perceptions** A good deal has been written about public perceptions of risks in general, and although the food and feed sectors have some distinctive features, many of the generalised research conclusions are directly relevant. It is apparent, that in all sectors of activity the public perception of risk reflects a complex integration and evaluation process in which the actual risk, defined in scientific terms, is only one of the factors taken into account. A number of researchers (see Craven and Johnson, 1999) have recorded non-risk factors influencing public perception, including those in Table 1.

**Table 1** Factors reducing v increasing the public perception of risk

| | | |
|---|---|---|
| - Voluntary v coerced | - Not dreaded v dreaded | - Individually controlled v controlled by others |
| - Natural v industrial | - Chronic v catastrophic | - Morally irrelevant v morally relevant |
| - Familiar v not familiar | - Knowable v unknowable | - Trustworthy sources v untrustworthy sources |
| - Not memorable v memorable | - Fair v unfair | - Responsive process v unresponsive process |

In the case of food, perceptions of risk and their impact on purchasing pattern are particularly complex. Firstly the consumer population appears to sub-set into a number of distinctive groups, whose perceptions are not necessarily the same. Secondly, factors such as price, taste, quality and convenience come into play; and concerns which are expressed about safety do not necessarily align with the factors that determine purchasing decisions. There are good examples of these phenomena in the market survey reports published on the web-sites of the Food Standards Agency (FSA) (www.foodstandards.gov.uk) and the Institute of Grocery Distribution (www.igd.com).

However, where the 'general rules' of public perception are manifest their impact on public attitudes to food-chain is very important. The evidence indicates that public perception tends to focus less on the scientifically determined risk and more on the non-risk factors (Table 1); there is a particular public preoccupation with 'catastrophic' events, which might impact dramatically on the lives of a large number of people. Thus, speed and severity of impact (particularly lethality) are important to perception, and the focus is on the 'disaster' that will occur if something goes wrong, rather than on the low statistical possibility of that event occurring. The public engages very quickly with some problems, e.g. *v*CJD or *E coli O157,* but it shows much less interest in problems which it regards as not life threatening, e.g. food safety in the home, or as not-urgent, e.g. healthy eating.

**Public communication** Traditionally in the UK, public communication on matters of food safety and public health was the domain of government alone. However, there has been a loss of public confidence in politicians and in government, a global business consolidation in the food manufacturing and retail sectors and the development of a myriad of food and environmental NGOs, campaign groups and other bodies. Together these changes have transformed the communication process since now the government is only one of many sources of public information.

There has been some recent improvement in the public's general perception of the food safety in the UK, resulting largely from a growth in consumer confidence in the FSA's openness and accessibility. However, much of the wider public debate continues to be characterised by divergent views on issues of food and feed safety and quality, or related topics. Some people argue that this is desirable, that the UK is adopting a 'democratic' information model, leading to well-informed consumers who make personal choices in their buying decisions. However, research suggests that only 10-12% of consumers adopt this approach, with the remainder either not being very interested or not being committed enough to spend time and effort evaluating the information available. This leads to a relatively volatile public communication environment in which an interested and actively engaged minority of consumers often sets the agenda that creates the 'headline issues'.

**Information providers** A further problem is that many of the information providers have a vested interest in advancing their agendas in the eyes of the public or of the government legislators. Thus, whilst the information placed in the public domain is not necessarily incorrect, it is often selective and presented in a way that 'makes a case': often, impartiality and objectivity are not high priorities. Authors, writing from very different background positions, have presented aspects of this market communication process as modern day 'conspiracies' (see Humphrys, 2001; North, 2001). However, an alternative interpretation is that it simply reflects a general institutional and corporate recognition of the power of the media and the potential force of public opinion on the political process. It is nonetheless disturbing to find that attempts to be 'media friendly' are resulting in the public misrepresentation even of the pronouncements of the most well respected scientific bodies, such as the Royal Society of London (Gilland, 2002).

**Supermarket dilemma** In view of the dominant retail position of the supermarkets and their huge influence on the whole of the food supply chain, it could be argued that the supermarkets should (some would argue already do) take the lead in public information provision. However, supermarket businesses are not scientifically well equipped for this role, and they are driven by the need to deliver shareholder profit and market growth, rather than impartial objectivity. Moreover, in a highly competitive market, individual companies will always seek to find market differentiation wherever it can be established; and offers of different or better food safety and quality assurance are an important part of marketing. In some instances supermarkets, whilst attempting to provide individual consumer assurance, may pursue policies that serve to amplify general public concerns. For example in non-GMO products and in organic foods, the supermarkets have responded to perceived 'market demand' without apparently considering the wider and longer-term food policy implications. With some justification, they might argue that they are in business to respond to consumer demand not to create public policy. However, since it is difficult to argue for the special benefits of one type of food production without denigrating the alternative, the supermarkets' policy decisions have a wide, and potentially subversive, effect on public perceptions of the safety and quality of the general food supply.

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# Nutrition and Production – the Scientist

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**Introduction**

Reducing costs for unit output, be it per kg liveweight gain, per kg carcass gain or per kg saleable meat yield will be essential for the future of the UK beef industry. Traditionally emphasis has been placed on reducing feed costs per unit output and data from MLC's Beefplan shows that between 65 – 82% of the variable cost of UK beef systems are accounted for by feed costs. However, with fixed costs being similar to total variable costs, it is also important to reduce the labour and machinery costs associated with the production of feed and feeding it. As far as nutrition/feeding of beef cattle is concerned, there are a number of areas where a reduction in costs can be achieved using existing knowledge but in other areas there are obvious gaps in knowledge which require further research. However, there appears to very little research into aspects of the nutrition/production of beef cattle going on, or being published, in the UK. (Only 8 out of a total of 228 papers presented at this conference are on nutrition/production of beef cattle.)

Effective diet formulation requires good information on the nutritive value of foods, the nutrient requirements of cattle and an accurate prediction of voluntary food intake. The majority of beef systems still rely on grass silage as winter forage. The use of NIR for evaluating both the nutritive value and intake characteristics of silage, coupled with least-cost computerised ration formulation programs using a modified UK ME system, gives diets which produce performance close enough to expectations in most situations. However, improvements to such programs to give better descriptions of the substitution rates of silage by the amount and type of supplement fed are required. Also required is a better description of the composition of live weight gain at various weights for the breeds/crosses now used in the UK. The current ME system overestimates the energy requirements of late maturing continental cross bulls, especially when these are taken to heavier weights. A co-ordinated effort is needed to develop improved nutritional programs for diet formulation for beef cattle but funding such a task would be a major problem. (A 'Feed into Beef' project similar to the 'Feed into Milk' that is funded by the MDC and industry would be a way forward!)

The UK Metabolisable Protein (MP) System was developed primarily for use in dairy cows but its use in its original form overestimates the ERDP and underestimates the DUP components of the protein requirement of beef cattle. This applies to some silage based finishing systems and also intensive systems of production. In both systems feeding excessive protein levels has been shown to increase carcass fatness which is undesirable, and is costly, wasteful and environmentally unfriendly.

Intensive finishing systems have become more popular as a result of low cereal prices. Such systems involve ad-libitum feeding from hoppers/bunkers thus saving on labour costs. Methods of reducing the costs of harvesting, preserving and processing cereals for feeding need to be evaluated. Whole crop wheat/barley, urea treatment of whole moist grain, crimping and harvesting with a forage harvester fitted with a mill are all options. The latter allows late harvesting of crops in virtually all weather conditions, either to produce conventional whole crop silage or, by cutting only the heads, a high energy feed which can be fed as a supplement or as the sole diet in intensive systems.

Although cereals are likely to be the major ingredient in intensive diets, the roles of high energy but fibrous by-products require evaluation to ensure that feed conversion ratio (FCR), and hence production costs, are minimised.

The patterns of performance in terms of liveweight gain, FCR and feed costs/kg LWG for various breeds/crosses need to be described so that better guidelines as to optimum slaughter weights are available.

Levels of protein in intensive diets are generally too high and savings can be achieved by feeding lower levels without compromising performance. Feeding systems in which low protein cereals and a protein supplement are offered free choice may be a way forward to reduce both feed costs and the labour and machinery required to mix diets. However, more work is needed to test whether this system works in all situations.

**Conclusions** Improvements in methods of intake prediction and estimating energy and protein requirements are required for incorporation into more effective diet formulation procedures. Ideally these improved systems should be made generally available for all to benefit but this may not happen for commercial reasons. Newer methods of cereal harvesting and storage should be evaluated as a means of reducing the cost, risk and hassle of getting the crop from 'field to trough'. However, obtaining funding to carry out this work will be a problem (or a challenge).

# Genetic Improvement of Beef Cattle -the Scientist

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**Introduction** For thousands of years, perhaps ever since the domestication of cattle, farmers have favoured, and therefore selected, certain animals above others in an attempt to create a population of beasts best suited to their needs. Over time the sophistication with which breeding cattle have been selected has improved as technology and understanding has developed. In Great Britain today the genetic improvement of cattle makes use of the three main methods available: breed substitution, crossbreeding and within-breed selection. This paper concentrates on the last of these but recognises the importance of the first two in commercial practice. For example there has been considerable substitution of native British beef breeds with continental breeds during the 1960s and 70s and there is continued use of crossbreeding on dairy cows and in suckler beef production. Within breed selection and performance recording is valuable for the continued improvement of pedigree beef cattle and as a means for commercial producers to select suitable herd sires.

**The national resource** Purebred beef animals constitute only a small proportion of the beef cattle population of Great Britain but have an essential role to play in the production of high genetic merit breeding bulls for crossing. The pedigree beef population is estimated to be around 80 000 cattle across more than 30 breeds. Through natural service and AI bulls from this population sire around 2 million calves a year. About 35 000 beef cattle are performance recorded, with 8 breeds accounting for 50% of performance recording.

'Beefbreeder' is the national pedigree beef evaluation scheme (run by Signet Farm Business Consultancy). It is designed to identify pedigree beef cattle with the genetic potential to produce calves that have good conformation and grow quickly. The system is designed to overcome the two difficulties that hamper genetic progress in Great Britain, namely the small herd sizes and the influence of management on performance. Without Beefbreeder, the small herd size often found in the UK pedigree beef industry means that the number of contemporaries in a herd, between which valid comparisons can be made, is low. This restricts the choices when making selection decisions and so limits the rate of genetic progress. A performance trait, like growth rate, is an expression of an animal's genotype. Management and the environment in which the animal lives influence performance traits. Without Beefbreeder comparisons between animals can only be made with confidence within herds (and then only between animals of approximately the same age, managed together) or within performance test groups. With Beefbreeder, comparisons can be made between individuals born in any herd, year or season.

**Best Linear Unbiased Predictor (BLUP)** In 1991 the statistical procedure known as BLUP - which has been used in the UK dairy industry since the early 1960s - was first used on data from pedigree beef herds in Britain following extensive MLC-funded research in SAC and the Roslin Institute. Essentially BLUP attempts to separate out the genetic factors influencing an animal's performance from the non-genetic (or 'environmental') factors such as management and feeding. In order to make this separation genetic links between contemporary groups are required. For example, at least one individual in a contemporary group must be the offspring of a sire who also has offspring in another contemporary group. Because of the wide use of AI in pedigree beef cattle links of this kind within breeds are good. Hence, related animals link small herds, and comparisons between herds become possible. BLUP compares related animals with their contemporaries across many different herds using the related animals as a benchmark because they share genes and are therefore expected to perform more similarly than unrelated animals.

The calculations are carried out using an individual animal model, which is the most sophisticated method of BLUP analysis available. What this means is that for each animal information on its own performance is used as well as all available data relating to the performance of its performance recorded relatives.

All the information relating to several recorded traits is then analysed *simultaneously* taking into account any correlations between traits. For example data on 200-day growth contributes to the evaluation of 400-day growth because the two traits are correlated. The degree to which each trait is inherited by the next generation (heritability) is also taken into account.

**Estimated Breeding Values** Estimated Breeding Values (EBVs) for each animal for each trait are then produced which are estimates of the genetic worth of the animal. The EBVs are termed multi-trait because they are calculated from information on all the measured traits. Because the influence of the environment has been accounted for the EBVs of all the animals in the breed evaluation can be directly compared, no matter which herd they belong to, which dramatically increases the size of the genetic pool from which replacements can be chosen. EBVs can also be compared across years, which allows genetic trends and progress to be monitored.

EBVs have the same units as the measured trait (e.g. kg for liveweight) and are expressed relative to a common baseline for all animals in the same evaluation. For all breeds in the UK the baseline is currently set so that the average of the breeding values for animals born in 1980 is zero. Accuracy values are presented along with the EBVs and provide a guide to the likelihood of an EBV changing (up or down) as more information on the animal and its relatives becomes

available. High accuracy values gives the commercial buyer confidence that animals with above average EBVs will pass these desirable characteristics on to their progeny.

Table 1 EBVs for nine recorded traits are currently produced:

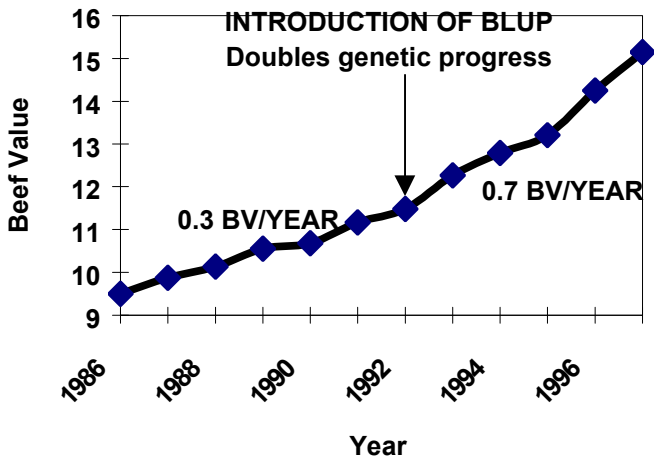
| EBV | Unit of measurement |
|---|---|
| ***Direct*** | |
| 200-day growth | kg |
| 400-day growth | kg |
| muscling score | recorded at 400 days on a 1-15 point scale |
| muscle depth | measured in mm by ultrasonic scanning at 400 days |
| backfat depth | measured in mm by ultrasonic scanning at 400 days |
| birthweight | kg |
| gestation length | days |
| calving ease | expected change in percentage of unassisted calvings |
| ***Maternal*** | |
| 200-day milk | kg |

**Multi-trait indexes** EBVs are usually presented for each trait measured which allows breeders to decide how much emphasis they wish to place on each trait in selection. However, they can also be combined into a multi-trait selection index for a specific breeding objective, in this case for growth and carcass traits, and selection can be based on this. It is widely agreed that this is the most efficient method of improving several traits at once. Economic weightings relevant to current market conditions are used to ensure each trait is given the appropriate amount of emphasis in the index. The aim of multi-trait index selection is to maximise the change in breeding value for the overall objective. This can be expressed as the sum of the breeding values for all of the traits in the breeding objective, each weighted by its economic value.

Two indexes are currently produced for each animal in the Beefbreeder genetic evaluation service, the Beef Value and the Calving Value. The objective of the Beef Value is 'to improve financial value of the carcass by genetically improving carcass weight, fat and conformation scores in line with current commercial carcass pricing structures'. It ranks animals on the expected carcass financial merit of their offspring. The objective of the Calving Value is 'to improve profitability by reducing the costs associated with difficult calvings (e.g. veterinary costs, loss of production). It is designed to aid producers to select terminal sires that will produce calves that are born easily.

**Benefits of using EBVs to aid selection** If all the traits in both indexes are recorded, 80% of the variation among bulls will be due to Beef Value traits and 20% to Calving Value traits. Selection on high EBVs for all traits will result in bulls leaving progeny with heavier carcasses of better conformation at a constant age, shorter gestation lengths and which are easier to calve than average.

**Genetic trends** Until about 1990 the improvements in carcass characteristics achieved were mainly because of the positive correlations between growth and carcass traits. Since then the introduction of BLUP and the wide use of muscle scoring and ultrasound scanning has made selection more accurate and speeded up the rate of genetic progress.




In the last 3 years the Beef Value has increased at over 0.7 units per year which is worth nearly £1 million per year to the beef industry through improved efficiency. Over five years that is worth around £14 million. Genetic improvement using BLUP techniques is a potent tool that adds lasting value to the beef industry. Its effects could be improved still further if more beef animals were recorded and the EBVs used more ruthlessly. The best breeders currently progress at over 1.5 Beef Value units per year.

To a commercial breeder and finisher the difference between using an average bull and a bull from the top 1% of the breed is worth around £30 per calf finished at current prices.

# Genetic improvements of beef cattle – the farmer/practitoner

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**Improves terminal sire line**

JSR Farms at Givendale runs a commercial cross-bred suckler herd of 100 cows alongside a pedigree Charolais herd of 110 cows. The Charolais breeding programme is focused on improving the output from the cross-bred herd by using BLUP to select sires with superior genetic merit for production traits. Breeding stock with high EBV's for growth and carcase traits are selected for re-breeding and attention is also paid to 200 day milk EBV's and to controlling the level of calving difficulty. Home-bred replacement heifers are selected with Beef Values ranked in the top 10% of the National breed and the stud of reference sires used in the programme have Beef Values in the top 1% of the breed.

As the result of this fast-track genetic selection process significant progress has been made to improving growth and carcase traits as shown by graphs 1 and 2.

The combined improvement of growth and carcase traits shows an accelerated rate of Beef Value achievement when compared with the National breed averages, and also shows two Beef Value unit's improvement per year (see graph 3).

From a practical point of view what do these genetic gains mean in terms of improved output from commercial Suckler cows?

For several years at Givendale I have been progeny testing bulls with Beef Value's ranked from the top 25% to the top 1% in the breed. A picture soon started to emerge of significant difference in the performance of the progeny. Calves sired by top 1% bulls grew faster and had better conformation than the calves sired by those bulls in the top 25% range for Beef Value.

Table 1
Progeny test results Givendale suckled bulls

| SIRES | 400 DAY WT KG | LW GAIN KG/DAY | CARCASE VALUE | DAYS TO SLAUGHTER |
|---|---|---|---|---|
| CH 40 | 592 | 1.48 | 660 | 414 |
| CH 35 | 570 | 1.42 | 631 | 410 |
| Difference | +22 | +0.06 | +31 | +4 |

In conclusion I would suggest that by selecting bulls with the highest beef values producers will significantly increase the output from their herds

**Improved Suckler Dam Line**

For years the dairy industry has supplied large numbers of beef-cross heifers as replacements for suckler herds. The heavily built Friesian, when crossed with our native beef breeds, produced highly fertile and efficient cows. They had the ability to maintain body condition on low-cost feeding regimes. They were robust, long-lived cows and their progeny finished to achieve high carcase specifications. The gradual expansion of Holstein bloodlines throughout dairy herds has resulted in dramatic changes to the type of animals now being used in suckler herds. These changes compromise efficiencies and therefore undermine profitability.
The disadvantages of Holstein-bred suckler cows can be summarised as follows: -

1) High-energy requirement – adding to feed costs.
2) Reduced fertility – prolonged calving periods.
3) Reduced longevity – increasing replacement costs.
4) Poorer progeny conformation – reducing income.
5) Excessive milk yields – leading to udder problems.
6) Reduced availability – UK dairy herd in decline.

Dairy-bred suckler cows are a by-product of high-yielding, large-framed, short-lived and poor conformation Holsteins. However, because of the simplicity of sourcing dairy-bred replacements there will be a continued demand for them.
It is more difficult to organise breeding programmes to produce efficient cross-bred beef cows using beef breeds with good maternal traits. The ideal cow-type should be of a medium size to control maintenance costs and should exhibit a

high level of reproductive ability, while retaining good beefing quality and longevity and adequate milk production. It is essential that these traits are consistently repeatable to maintain high levels of output.

**All commercial suckler cows should be crossbreds**.
Extensive trials in the USA have proved that crossbred cows and calves perform better than do pure-breds. The hybrid vigour generated by cross-breeding improves those traits with low-heritability such as fertility, calving ease, calf survival, milking ability, cow longevity and early puberty. All these traits combine to increase herd output by 22 % when compared with the output of the pure-breeds, which make up the cross-bred.

So how do we go about organising a breeding programme to produce heifers of the desired type? Members of The Beef Improvement Group (BIG) started a rotational cross-breeding programme in 1996 using South Devon and Angus bulls on existing cows. The plan was to retain the heifer calves and mate them to the two breeds alternately, i.e. rotational cross breeding. Members of BIG then went to the USA in 1997to visit the USDA Meat Animal Research Centre (MARC) in Nebraska and the Leachman Cattle Company in Montana. The outcome of that and several subsequent visits has led BIG to start a composite breeding programme in the UK using USDA MARC technology and Leachman genetics. Animal scientists at MARC have been working with 7000 cows for the last 30 years to develop more efficient methods of breeding high-output cow types. They have combined the differences of several breeds to create composite breeds with high levels of uniformity and reproductive efficiency. Their results have shown that composite breeding offers a procedure that is more effective than continuous cross-breeding for utilising genetic differences among breeds to achieve and maintain optimum performance levels for economic traits on a continuing basis, while retaining 75 % of the hybrid vigour generated by the first cross (F1). BIG has established a nucleus herd of the four-breed composite (MARC 2) known as the Leachman Stabiliser.

Table 2
Effect of cow breeding on performance

| | Cont x Dairy | Pure Cont | Rotation | Composite |
|---|---|---|---|---|
| Lifetime Cow output (kg weaned calf/cow | 1201 | 975 | 1620 | 1914 |
| Calf Value (p/kg) | 85 | 90 | 87 | 87 |
| Margin per cow | 15.04 | 17.23 | 57.02 | 65.02 |

Table 3
Effect of cow breeding on performance

| | Cont x Dairy | Pure Cont | Rotation | Composite |
|---|---|---|---|---|
| Maintenance (MJ/d) | 79 | 75 | 68 | 68 |
| Calves / Cow | 5 | 5 | 7 | 8 |
| Fertility % (Calved/cow 12 week bulling) | 89 | 78 | 89 | 92 |
| Cost of calf (p/kg) | 78.7 | 80.6 | 62.4 | 59.8 |

In conclusion it would appear that composite breeding offers a technique, which will provide an opportunity to increase output from specifically designed cow types. However large numbers of animals are required to build a sustainable programme to avoid in-breeding and this can only be achieved by breeders co-operating in an organised way.

## Meat Quality

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The relationship between livestock producer and meat plant operator has always been one of suspicion. Now, as a result of last year's tragic Foot & Mouth outbreak, it is the belief of the Speaker that to use this period of recuperation and rebuilding to develop solid relationships and meaningful trust between both parties. We have a shared challenge if Britain is to remain with a strong meat and livestock sector and not simply rely on the requirements of the consumer to be increasingly met by product from imported sources.

Britain will never be the cheapest producer of beef and lamb but we must aim to restore the credibility of British meat to one which is again respected throughout the international scene. This will require development of niche markets with both sectors within the industry striving together for efficiency and strength.

Producer and plant operators need to fully appreciate and understand each others problems so communication of market requirements and conditions becomes mandatory.

As a meat processor, we have to ensure that we provide consumers with what they want, and in the form they want. This will call for investment, sensible use of technology and constant attention to food safety.

The Speaker intends to share with the audience the findings of a survey conducted by his Company in the north of England as to what butchers and their customers look for when they buy meat. There are important issues here if we are all together to strengthen the market for meat and meat products and mount a sustained effort to provide a consistent and quality meat eating experience every time.

**Meat Quality**

Professor Jeff Wood
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Two terms characterise the carcass and meat: carcass quality and meat quality. The former describes the yield of meat and the ratio of lean to fat and the farmer is paid for it, more directly in pigs. Meat quality describes the appearance and taste of meat and although these also vary and are arguably more important to consumers than yield, the industry does not obviously recognise this variation in terms of price differentials. Reasons for this include the difficulty of deciding where in the production-processing chain quality variation is introduced and the lack of reliable, easily-used measuring systems.

The talk will describe economically important aspects of meat quality and the reasons for the variation which is commonly observed. Variation can arise on the farm (eg toughness in young bulls, better shelf life due to high levels of vitamin E or pale pork because of the halothane gene) or during processing (eg slow chilling and longer conditioning times produce tender meat). The best way to improve meat quality is for producers and processors to work closely together to provide reliably higher quality for retailers.

Breeding and feed companies, should be part of these arrangements and all partners share higher prices arising from consumers willingness to pay for demonstrably better products and less wastage due to longer shelf life and lower colour variation.

One reason these schemes have not been widely introduced in the UK is that quality improvements are often costly or benefit only one of the production-processing partners. The confrontational nature of food chain relationships prevents a truly integrated approach. However, as quality moves up the agenda, more productive partnerships are being established. These developments would benefit from more direct measurement of quality parameters (eg muscle pH and colour) and simpler measuring devices.