

*Proceedings  
of the  
British Society  
of Animal Science*

*2003*

Published by  
*British Society of Animal Science*

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*Proceedings  
of the  
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*The Proceedings of the British Society of Animal Science constitute summaries of papers presented at the Society's Annual Meeting in York in March 2003*

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ISBN 0906562 41 4

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The British Society of Animal Science is extremely grateful to the following organisations who have generously supported the Annual Meeting 2003



# Induction of ovulation with GnRH or oestradiol benzoate lowers plasma progesterone concentration within the first week of ovulation in non-lactating Holstein cows

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**Introduction:** Oestrous synchronisation involves synchronisation of ovarian follicular turnover, new wave emergence, and finally induction of ovulation which can be achieved with an injection of either GnRH (Pursley *et al.* 1997) or oestradiol benzoate (ODB) (Day *et al.* 2000). A comparative study investigating corpus luteum (CL) and follicular emergence after the administration of either GnRH or ODB at pro-oestrus has not been reported. It was hypothesised that the injection of ODB at pro-oestrus would delay emergence of the first post-ovulatory follicular wave, but that CL development and plasma progesterone concentrations would be similar in cows induced to ovulate with either GnRH or ODB.

**Materials and Methods:** Non-lactating cycling dairy cows (n=14) were randomly divided into three groups (group A, n=5; group B, n=5; group C n=4). Every cow was pre-synchronised with an injection of PGF2 $\alpha$ . On Day -10 (Day 0=ovulation), a CIDR device was inserted into the vagina of each cow before it was injected i.m. with 2mg ODB. The CIDR device was withdrawn on Day -2, followed by an i.m injection of 25mg PGF2 $\alpha$ . Every cow in Group A was injected i.m with 1mg ODB on Day -1; and those cows in Group B with 250 $\mu$ g GnRH, i.m. while those in Group C received no other treatment during pro-oestrus. Ovarian structures were investigated every day from Day -5 until Day 10 while daily blood samples were collected from every cow during the same period for hormonal analysis. The procedure was repeated over three rounds in a crossover design. A cross over design was used to analyse all rounds combined, the effects being sequence vs animal within sequence, round vs residual and treatment vs residual.

**Results and Discussion: First wave follicular development:** The emergence of a new follicular wave was delayed when cows were treated with ODB ( $0.8 \pm 0.2$  days vs  $1.7 \pm 0.9$  days vs  $0.8 \pm 0.9$  days in control, ODB and GnRH treatment groups respectively,  $p=0.02$ ). Both the interval from ovulation to peak plasma oestradiol concentration and the peak oestradiol concentration associated with the first follicular wave were similar amongst the three treatment groups ( $5.7 \pm 0.3$  days vs  $5.5 \pm 0.2$  days vs  $5.3 \pm 0.2$  days for control, ODB and GnRH cows respectively,  $p=0.45$   $1.4 \pm 0.2$  pg/ml, vs  $1.6 \pm 0.2$  pg/ml vs  $2.0 \pm$  pg/ml for control, ODB and GnRH groups respectively,  $p=0.10$ ).

**Corpus luteum development and function:** The CL was identified sooner in the untreated controls ( $2.6 \pm 0.3$  days vs  $3.1 \pm 0.2$  days vs  $3.3 \pm 0.2$  days for control, ODB and GnRH treatment groups,  $p=0.02$ ). The rise in plasma progesterone concentration between Days 3 and 9 was higher in controls ( $1.22 \pm 0.12$  ng/ml/day vs  $0.88 \pm 0.06$  ng/ml/day vs  $0.87 \pm 0.10$  ng/ml/day for control, ODB and GnRH treatment groups respectively,  $p=0.02$ ) so that average progesterone concentration when cows were untreated was higher on Day 9 ( $5.0 \pm 0.3$  ng/ml vs  $3.6 \pm 0.2$  ng/ml vs  $3.6 \pm 0.4$  ng/ml for control, ODB and GnRH treatment groups respectively,  $p=0.02$ ).

Treating non-lactating Holstein cows with either 1mg ODB or 250 $\mu$ g of GnRH during pro-oestrus influenced the development of the subsequent CL. Cows treated with either 1mg ODB or 250  $\mu$ g GnRH had a significantly smaller CL on Day 10; this CL secreted less progesterone than the CL formed after cows ovulated spontaneously. This consistent association of the treatment groups with the smaller CL strongly suggested an effect of induction of ovulation 24 or 36 hours after CIDR device removal with ODB and GnRH respectively.

**Conclusion:** The injection of ODB may have delayed the emergence of the first follicular wave after ovulation; administration of ODB or GnRH lowered the progesterone rise so that the maximum dioestrous concentration of progesterone on Day 9 was lower when cows were treated during pro-oestrus. The significance of these findings on the fertility of dairy cows needs further evaluation. The effect of this induction on the CL development after Day 10 needs to be studied. .

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# Tissue-specific differences in insulin receptor m-RNA isoform ratio in two dairy cow breeds

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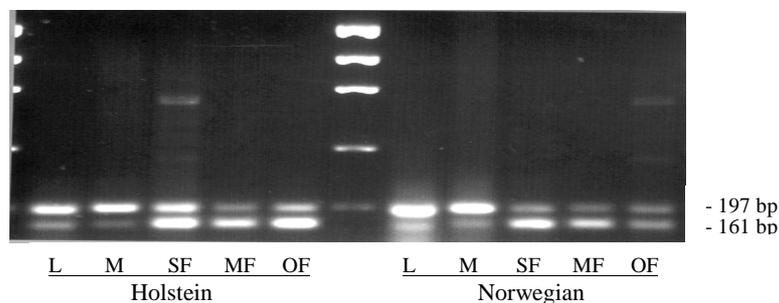
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**Introduction** The insulin receptor (IR) is one participant in the partitioning of absorbed nutrients to, and between, the insulin-responsive tissues of animals. We previously reported the alternative splicing of a 36 base pair exon 11 segment of the sheep IR gene and found differences in the ratio of the resultant 11<sup>-</sup>m-RNA and 11<sup>+</sup>m-RNA isoforms between muscle and fat depots in crossbred, but not purebred, Texel lambs (McGrattan *et al.*, 1998). Because the IR isoforms, when expressed in cultured cell lines, differ in affinity for insulin by two fold or more (McClain, 1991), variations in the ratio of IR isoform expression may have practical significance for the partitioning of nutrients between tissues and thus for differences in lean and fat gain between the purebred and crossbred lambs. Similarly, variation in IR m-RNA isoform ratios amongst the adipose depots in different breeds of dairy cow could have practical significance for differences in the ability of the breeds to store and mobilise energy and thus for differences in lactational performance between dairy breeds. This study is a preliminary investigation of this hypothesis.

**Materials and methods** Total RNA was extracted from bovine liver and subjected to reverse transcription PCR (RT-PCR) against two ovine specific IR primers generated by McGrattan *et al.* (1998). PCR products were separated on 2% agarose gels and nucleotide sequenced to ascertain the degree of homology with the comparative portion of the ovine IR. Subsequently, these same primers were used to probe, again by RT-PCR, RNA extracted from samples of liver (L), *longissimus dorsi* muscle (M), spleen (S) and subcutaneous fat (SF), omental fat (OF), renal fat (RF) and mesenteric fat (MF) from second parity, late lactation, Holstein-Friesian and Norwegian cows (n=3 each) fed on identical diets. The samples were taken into liquid nitrogen within 20mins of slaughter and stored at -80° until RNA was extracted and analysed. The percentage abundance of the IR m-RNA isoform PCR products was determined by scanning densitometry and differences between breeds were statistically analysed by analysis of variance.

**Results** Gel electrophoresis of cDNA RT-PCR amplification products of bovine liver indicated two discrete bands differing in size by 36 base pairs (bp). The nucleotide sequence of the probed area was 98% homologous to the comparable ovine sequence. A densitometric scan of the IR mRNA isoform distribution in the tissues from one cow of each breed is shown in Figure 1. The mean percentage abundance of the 11<sup>-</sup> IR mRNA isoform is shown in Table 1. The relative abundance of either isoform in all four fat depots was different from that for liver, muscle and spleen. However the relative abundance of the mRNA isoforms differed between the two breeds only in omental fat (P<0.05).

**Figure 1.** A densitometric photostan of the two mRNA isoforms of the bovine IR extracted from liver (L), muscle (M), and subcutaneous (SF), omental (OF) and renal (RF) fat of one Holstein (H) and one Norwegian (N) cow



**Table 1.** Mean relative abundance (%) of the bovine IR 11<sup>-</sup> mRNA isoform in selected tissues from Holstein and Norwegian cows

Tissue	L	M	SF	RF	OF	MF	S
<b>Holstein-Friesian</b>	36.5	21.6	57.8	59.3	62.4	64.3	80.1
<b>Norwegian</b>	31.9	17.5	52.7	57.1	55.6	61.0	78.8
<i>Significance of breed</i>	NS	NS	NS	NS	P<0.05	NS	NS

**Conclusions** The presence, in all tissues, of two discrete PCR products differing by exactly 36 bp demonstrates that alternative splicing of the exon 11 region of the IR gene occurs in the bovine animal as in ovine, human and all other species so far studied. Differences between tissues in the relative percentage abundance of the 11<sup>+</sup> and 11<sup>-</sup> isoforms provides evidence of tissue-specific regulation of alternative splicing of the IR gene in the bovine animal also. The observation of a significant between-breed difference in the relative abundance of the IR mRNA isoforms in omental adipose tissue only, amongst the four adipose tissues investigated, may have physiological relevance, given the important role of omental fat in the storage of energy for early lactation in dairy cows.

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# Effects of rearing regime on milk production and metabolic hormone concentrations in high genetic merit Holstein-Friesian dairy heifers

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**Introduction** Nutrition during the rearing period has significant effects on subsequent milk production and reproductive performance of dairy herd replacements. Carson *et al.* (2002) reported that heifers reared to calve down at 620 kg, in contrast to 540 kg live weight, produced 11% more milk, lost more weight and body condition score (BCS) post-calving and had a 30 day longer calving interval. This suggests that a higher BCS at calving and/or a greater rate of BCS loss during lactation appear to be correlated with poorer fertility. The objectives of this experiment were to investigate the effect of (1) diet composition during the rearing period and (2) live weight at first calving on body size and condition score changes during the first lactation and to assess linkages with metabolic hormone concentrations.

**Materials and Methods** Eighty Holstein Friesian heifers [PTA (2000) for fat and protein yield 21.7 (s.d.7.47) kg] were used in the study. The mean initial age of the heifers was 82 (s.d. 26.1) days and mean live weight was 88 (s.d.22.8) kg. The study used four rearing treatments in a 2 (target weight at first calving) X 2 (diet type) factorial design. Animals within each treatment were balanced for source of animal, live weight and genetic merit. Treatments consisted of two pre-calving live weights, either 540 or 620 kg, and within these half of each were offered grass-based diets during the summer and grass silage-based diets during the winter periods, and the other half were offered straw/concentrate based diets during the winter and the first summer periods and a grass-based diet in the second summer. Mating began at 14 months of age by artificial insemination, with the aim of calving heifers on each of the treatments at 2 years of age. Live weights and BCS were recorded fortnightly during the rearing period, and weekly during lactation. A representative sample of 16 animals (4 from each treatment) were selected for serial blood sampling for circulating metabolic hormone concentrations 3 months post calving. An indwelling sterile jugular catheter was inserted prior to sampling, a pre-prandial serum sample was taken and after feeding 5ml serum samples were taken every 15 min for 9 hours. The hourly samples, including the pre-prandial sample, were analysed for insulin using a double antibody radio-immunoassay (RIA) while composites of hours 1-3, 4-6 and 7-9 samples and the pre-feed sample were analysed for blood metabolites, IGF-1 and leptin (both by RIA). Data were analysed using a REML (restricted maximum likelihood) analysis with diet, calving live weight and all interactions applied as fixed effects.

**Results** During the first 3 months of lactation heifers reared to calve at 620 kg produced more milk than animals reared to calve at 540 kg ( $P<0.05$ ) (Table 1). Diet type during the rearing period had no significant effect on milk yield over this same period. Live weight and BCS loss during early lactation was greater for the heifers reared to calve at 620 kg ( $P<0.001$ ). The nadir of live weight and BCS was reached two months post-calving for animals reared to calve at 540 kg and 3 months post-calving for animals reared to calve at 620 kg. Circulating levels of serum non-esterified fatty acids (NEFA) were higher in animals reared on silage-based diets compared with those reared on straw-concentrate based diets. In addition, NEFA levels were higher in animals reared to calve at 620 kg ( $P<0.01$ ). Heifers reared to calve at 620 kg had significantly higher concentrations of serum leptin compared with animals reared to calve at 540 kg ( $P<0.001$ ). In addition, animals reared on silage-based diets had higher leptin concentrations than those reared on straw-concentrate based diets ( $P<0.05$ ). A strong relationship was observed between leptin concentration and BCS at first calving ( $P=0.052$ ) ( $\text{leptin}=1.7904 \times \text{BCS}-0.4502$ ,  $R^2=0.84$ ) while live weight at first calving and diet type during the rearing period had no significant effects on insulin or IGF-1 concentrations in month three of lactation.

**Table 1** Effects of diet type during the rearing period and weight at first calving on milk production, live weight change and metabolic hormone concentrations during the first 3 months of lactation

	Rearing diet (winter period)			Pre-calving live weight (kg)			Significance	
	Silage	Straw	s.e.d.	540	620	s.e.d.	Diet	LWT
Cumulative milk yield (0-3mo)(kg)	2357	2260	61.2	2235	2382	58.4	NS	*
Liveweight change (kg)	-63.1	-77.1	7.06	-44.4	-95.8	6.65	NS	***
Condition score change	-0.27	-0.25	0.053	-0.15	-0.37	0.05	NS	**
NEFA† (meg/L)	0.18	0.12	0.021	0.12	0.18	0.021	**	**
Leptin† (ng/ml)	4.25	3.88	0.142	3.75	4.38	0.142	*	***
IGF-1† (ng/ml)	183.5	206.0	14.75	186.6	202.9	14.75	NS	NS
Insulin† (µU/ml)	14.91	17.69	2.723	16.15	16.44	2.723	NS	NS

† Samples collected from 16 representative heifers @ month three of lactation

**Conclusions** Milk yield in early lactation was increased by rearing heifers to heavier weights at first calving. The increase in milk yield in heifers reared to calve at 620kg compared with 540 kg was supported through an increased mobilisation of body adipose reserves as suggested by the greater BCS loss, higher serum NEFA concentrations and higher serum leptin concentrations in early lactation. Diet type during the rearing period had a significant effect on leptin concentrations but had no significant effect on milk yield.

## References

Carson, A.F., Dawson, L.E.R., McCoy, M.A., Kilpatrick, D.J. Gordon, F.J. 2002) Effects of rearing regime on body size, reproductive performance and milk production during the first lactation in high genetic merit dairy herd replacements. *Animal Science* **74**: 553-565.

## Seasonal tissue changes in Scottish Blackface ewes over multiple production cycles

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**Introduction** A previous study using X-ray computed tomography (CT) of Scottish Blackface hill ewes found that carcass fat, internal fat and muscle are depleted during pregnancy and early lactation and deposited during late lactation and the dry period. Relationships were also found between fat and muscle levels and lamb production traits (Lambe et al., 2002a). Changes in tissue levels over multiple production cycles of the hill ewe, and how these changes relate to lamb production are also of interest. The aims of this study were to model changes in carcass fat, internal fat and muscle through three production cycles and to compare patterns of tissue change in ewes producing different numbers of lambs.

**Methods** Scottish Blackface ewes (n=271) were CT scanned four times per year: pre-mating, pre-lambing, mid-lactation, weaning, from pre-mating at 2-years-old to pre-mating at 5-years-old. Prediction equations (Lambe et al., 2002b) were used to estimate total weights of carcass fat, internal fat and muscle from cross-sectional CT images, for each animal at each scanning event. Predicted tissue weights were modelled using random regression analyses, where sin / cos curves were fitted within ewe using ASREML (Gilmour et al., 2001) for each of the three tissues, as a function of ewe age in days. Fixed effects of number of lambs born, number of lambs weaned the previous year, two- and three-way interactions between these two traits and age, and year of birth of the ewe were fitted. Residual error was split into four classes, representing the four scanning events, assuming residual error to be homogeneous within class, but heterogeneous across classes. Correlations between solutions predicted by the model and CT tissue weights, for each animal at each event, were calculated to assess the fit of the model. Animal solutions obtained from the analysis were used to calculate average fortnightly phenotypic values (weights of carcass fat, internal fat and muscle) for animals with a given numbers of lambs. Solutions were averaged over different number of lambs born the previous year and years of birth of the ewe, to remove variation due to these fixed effects. Differences between different levels of the fixed effects of litter size and the interaction between litter size and age of ewe were tested for significance.

**Results** Correlations between CT tissue weights and those predicted by the model were 0.87, 0.83, 0.87 for carcass fat, internal fat and muscle respectively. Figure 1 shows mean animal solutions for tissue weights, smoothed over time. Fat and muscle were mobilised in times of energy deficit and deposited in times of surplus energy. Fat levels increased with age in barren ewes, but not in ewes that produced lambs. Muscle weight increased in all ewes with age. Differences in carcass fat level between barren ewes and ewes with lambs (single/twin) increased significantly ( $P < 0.05$ ) over time (age of ewe-litter size interaction). This difference was also observed, but was not significant, in internal fat and muscle ( $P > 0.05$ ). Preferential management of twin-bearing compared to single-bearing ewes may explain similar tissue levels.

**Conclusions** Seasonal weight changes in fat and muscle in Scottish Blackface ewes can be modelled successfully over multiple production cycles using sinusoidal functions and random regression methodology. The pattern of carcass fat depletion and repletion over time in ewes rearing lambs is significantly different from that of ewes without lambs. Significant differences were not found in levels of internal fat and muscle over time between ewes producing different numbers of lambs. The energy required for reproductive function and lactation throughout the lifetime of the ewe is met by mobilisation of fat to a greater extent than muscle, resulting in no net aggregation of fat in reproducing ewes.

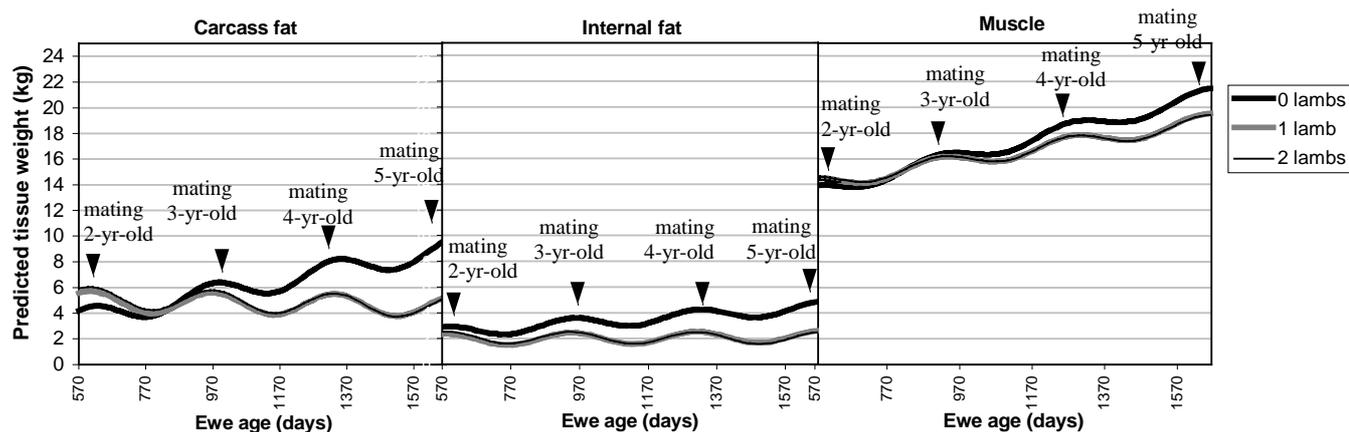


Figure 1 Tissue changes over multiple production cycles in ewes producing different numbers of lambs

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**Acknowledgements** Thanks to MLC, SEERAD and BWMB for funding and Kirsty McLean for data collation.

# Breed and parity differences in ovine placentation: Implications for placental efficiency and lamb vigour

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**Introduction** Lamb vigour at birth (i.e. time to stand after delivery) is known to be affected by a number of maternal factors, such as parity and litter size (Dwyer, 2002), as well as lamb breed (Dwyer et al., 1996), and is related to subsequent lamb survival (Dwyer et al., 2001). It is likely that these factors act before birth to influence lamb development and may exert their effects through differences in placental development and function. The purpose of this experiment was to examine the effects of ewe parity, litter size and ewe breed on placental components, and relate these to lamb behavioural development at birth.

**Materials and Methods** Ninety-three placentae were collected from 35 Scottish Blackface ewes and 26 Suffolk ewes, over 2 years (32 ewes contributed records in both years). Ewes were aged between 2 and 4 years at lambing and were in parity 1, 2 or 3. There were 38 singleton, 46 twin and 9 triplet pregnancies. Lamb behaviours were recorded for the first 2 hours after birth. Placentae were collected immediately after delivery (approximately 2 hours after lamb birth), cleaned and weighed. Individual placental cotyledons were dissected from the chorioallantois and sub-divided by size (small < 1cm diameter; medium between 1-5 cm; large > 5 cm). The number of cotyledons in each size class was counted and mean weight determined. Lamb birth weight and crown-rump length were measured at 24 hours after birth. The factors influencing placenta weights and components were investigated using the Restricted Maximum Likelihood procedure. The relationship between placental efficiency (defined as g of lamb produced per g of placenta) and lamb vigour (nlog time to stand) were investigated by regression analysis in singleton pregnancies.

**Results** Litter weight as a percentage of maternal weight increased with parity (Table 1) and litter size ( $P < 0.001$ ), and was higher in Blackface than Suffolk ewes (Table 1). Placenta weight was significantly correlated with litter weight ( $r^2 = 44.6$ ,  $P < 0.001$ ), but not lamb length, and increased with ewe parity (Table 1) and litter size ( $P < 0.001$ ). Suffolk ewes had heavier placentae than Blackface ewes (Table 1). However, placental efficiency was greater in Blackface ewes (weight lamb/weight placenta: Table 1). Cotyledon number was greater in Blackface ewes than Suffolks (Table 1), and was lower in singleton pregnancies than other litter sizes (mean cotyledon number: singleton = 74.07, twin = 90.83, triplet = 89.68; s.e.d. = 8.66,  $P < 0.05$ ). Average cotyledon weight increased with ewe parity ( $P < 0.001$ ) and litter size ( $P < 0.001$ ) and was greater in Suffolk than Blackface ewes (Table 1). The sire of the litter also had a significant influence on average cotyledon weight ( $P < 0.005$ ). All-male litters had heavier cotyledons than all-female or mixed sex litters (male = 1.88, female = 1.76, mixed = 1.78, s.e.d = 0.24,  $P < 0.01$ ), there were no other effects of litter sex. In singleton lambs there was a significant negative relationship between placental efficiency and lamb time to stand ( $b = -0.413$ ,  $r^2 = 17.1$ ,  $P < 0.05$ ), suggesting that a low placental efficiency was associated with lambs that were slow to stand.

**Table 1.** *Effects of parity and breed on placental parameters*

	Parity 1	Parity 2	Parity 3	s.e.d.	P	Blackface	Suffolk	s.e.d	P
Litter wt%	11.00	12.38	13.75	0.69	<0.001	14.48	10.27	0.78	<0.001
Placenta wt (g)	400.8	536.3	647.6	73.11	<0.05	468.0	588.4	66.2	<0.001
Placental efficiency	15.38	18.00	19.89	2.28	=0.074	19.40	16.12	2.27	<0.05
Cotyledon wt (g)	1.35	1.83	2.26	0.21	<0.001	1.39	2.24	0.19	<0.001
No. cotyledons	88.25	79.84	86.49	10.37	NS	93.70	76.02	10.36	<0.05

**Conclusions** Blackface ewes appeared to have more efficient placentae, which may be related to an increased number of placental cotyledons. Suffolk ewes appear to compensate for their lower number of cotyledons by increased cotyledon size, although this may be at the expense of placental efficiency. The unexpected effect of sire on cotyledon weight suggests an indirect mechanism for sires to increase the birth weight of their progeny. The preliminary data suggest that lamb vigour at birth may be impaired in ewes with less efficient placentae.

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## Do cows regulate diet choice within the short-term time frame of a meal?

M. P. Yeates, B. J. Tolcamp and I. Kyriazakis

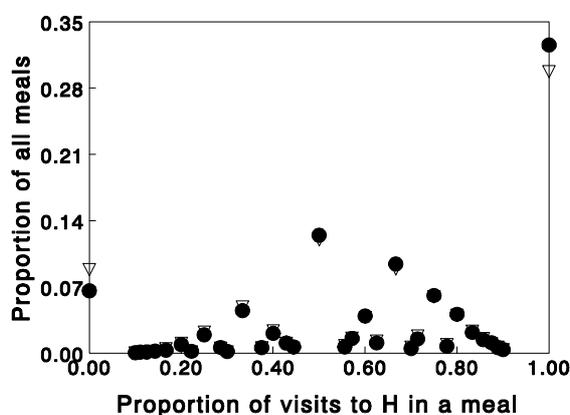
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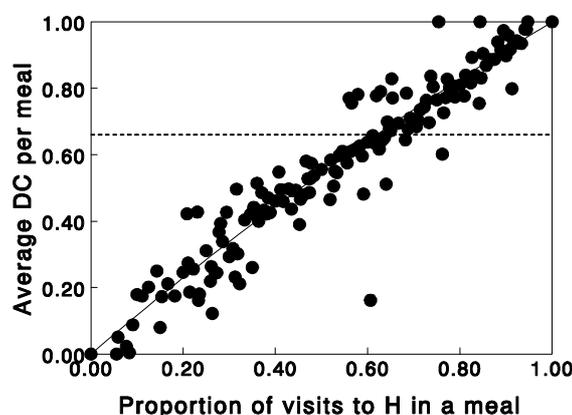
**Introduction** When cows are offered a choice of foods they are able to select a consistent combination of these foods over long periods of time. Consistent long-term diet choice (DC) is the result of feeding behaviour, which may be regulated in the short-term. The shortest unit of feeding that can be measured is often a visit to a feeder supplying one food type only. These visits are usually clustered into meals, which are the shortest biological unit in which DC can be expressed. Previous work led us to hypothesise that animals may select a consistent diet within meals, thus ensuring nutrient synchronisation in the short-term. Therefore, the aim of this study was to investigate whether long-term average DC was a direct result of cows selecting a consistent diet within meals.

**Materials and Methods** This study used data collected from 16 dairy cows. Cows were offered a choice of high (H) and low (L) protein foods that consisted of 70% grass silage and 30% concentrate on a fresh weight basis, via 12 computerised food dispensers. Visits were clustered into meals after estimating individual meal criteria (i.e., the longest non-feeding interval, between visits to a feeder, which can be considered part of a meal) using the method of Yeates *et al.* (2001). For each meal, the proportion of visits to H feeders was calculated. The observed frequency distribution of meal composition, in terms of the proportion of visits to H feeders, was determined. Subsequently, observed visits were randomly re-clustered into bouts and the frequency distribution of random bout composition was calculated. This was used to test whether the observed frequency distribution of meal compositions differed from the frequency distribution of bout compositions. If cows regulated DC within meals then we would expect many more meals with a composition similar to the long-term average DC than predicted by the composition of random bouts. We also investigated if cows regulated DC by adjusting their intake per visit depending on the food type visited. If this occurred we would expect many meals to have a similar DC to the long-term average, irrespective of the proportion of visits to H in the meal. This was investigated by comparing the observed DC with that expected if DC, was, or was not, regulated within meals.

**Results** Cows ate a diet consisting of proportionally  $0.7 \pm 0.02$  kg of H per kg of intake, i.e. significantly different from random ( $P < 0.001$ ). The average meal criterion was  $25.6 \pm 1.47$  min, which resulted in an average  $6.0 \pm 0.21$  meals per day. Figure 1 shows that the frequency distributions of meals and random bouts coincided. Indeed, there were not more meals with a composition similar to the long-term DC than predicted by random bouts. This provided no evidence that cows attempted to achieve their long-term average DC within meals. Figure 2 shows that there was no evidence that animals attempted to regulate DC within meals by adjusting their intake per visit ( $P > 0.8$ ). Indeed, the observations are clustered around the expectations for animals that do not regulate DC on a meal basis.



**Figure 1** Proportion of all meals that have a given proportion of visits to feeders supplying H in a meal. The observed (●) and predicted (▽) frequency distribution of meal or bout compositions, respectively.



**Figure 2** Average diet choice in relation to proportion of visits to H feeders in meals. Dots represent the observations. The expected diet choice if this is (---), or is not (—), regulated within meals.

**Conclusions** Analyses have shown that these cows, which selected a consistent non-random long-term diet, did not attempt to achieve this within the short-term time frame of meals. The importance of supplying diets that synchronise nutrient supply can therefore be questioned. This work has implications for our understanding of how animals monitor and regulate their nutrient balance and consequently for the nutritional environment that we should place animals in.

**Acknowledgements** This work was funded by BBSRC, BOCM-PAULS and SEERAD.

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# Consequences of variation in feeding behaviour for the probability of cows starting a meal as estimated from pooled data

M. P. Yeates, B. J. Tolcamp and I. Kyriazakis

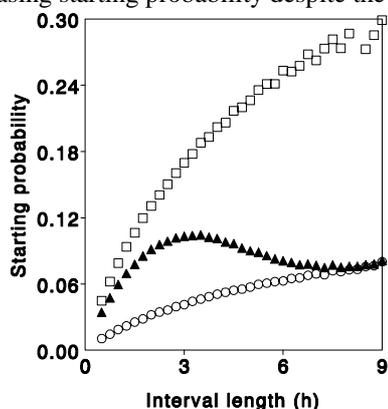
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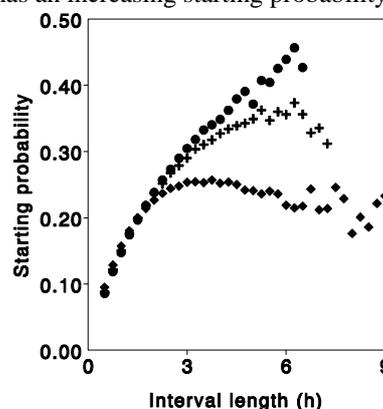
**Introduction** The analysis of short-term feeding behaviour may give insights into how food intake is regulated in farm animals. Food intake is often recorded in terms of feeding events, e.g. visits to feeders, which can be clustered into meals. This enables calculation of the probability of cows starting a meal in relation to time since the last meal, which is thought to give insight into intake regulation. Starting probabilities are often calculated after data have been pooled, e.g. across day and night or across individuals. Recent work suggested that such pooling might have strongly affected previously published conclusions. We therefore constructed simulation models to investigate how such pooling affects interpretation of feeding behaviour and consequently the biological significance attached to results.

**Materials and Methods** Simulation models were based on the assumption that starting probabilities would increase with time since the last meal, in agreement with the biological principle of satiety. Models were parameterised using information from Tolcamp *et al.* (1998). In this study data were collected from 16 dairy cows which averaged six meals per 24 h (individual variation resulted in a proportional CV of 0.14), with proportionally 0.59 of these during the day. Individual starting probabilities (the probability that a cow will start feeding within the next 15 min after a given non-feeding interval) were found to increase with time since the last meal. In the first model, the consequences of pooling across day and night were investigated with simulated data by systematically varying the average number of meals per 24 h and the proportion of these meals occurring during the day. In the second model, the population mean of the average number of meals per 24 h and the CV were varied systematically to explore pooling of data across many simulated individuals. In this way, we explored the consequences of pooling for starting probability interpretation.

**Results** Simulation modelling showed that pooling across day and night had no serious consequences when diurnal variation was low (i.e. cows ate a similar number of meals during the day and night). However, when diurnal variation was higher (i.e. Figure 1) then pooling across day and night (both with an increasing starting probability) could lead to a decreasing starting probability. Figure 2 summarises the output from the model simulating pooling across individuals which vary in their average number of meals per 24 h. When this individual variation is low then pooling is of little consequence and the starting probabilities continue to increase with time. However, as individual variation increases then the effect of pooling across individuals becomes more evident. This results in, first an increasing and subsequently a decreasing starting probability despite the fact that every individual has an increasing starting probability.



**Figure 1** Starting probability in the next 15min in relation to the length of the preceding non-feeding interval, calculated from the model simulating day (□), night (○) and pooled day and night (▲). Simulated for an average of six meals per 24 h and proportionally 0.7 of meals occurring in the day.



**Figure 2** Starting probability in the next 15min in relation to the length of the preceding non-feeding interval, as calculated from the model simulating pooling across individuals. Simulated for a population mean in the average number of meals per 24 h of 12 and proportional CV of 0.0 (●), 0.1 (+) and 0.2 (◆).

**Conclusions** Simulation has shown that pooling can result in starting probabilities that decrease with time even if the un-pooled data has an increasing starting probability. Inappropriate pooling could therefore lead to misinterpretation of experimental data. This may explain why the results of some published studies seem not to agree with the biological principles of satiety. The problem of pooling should be recognised when attempting to understand intake regulation.

**Acknowledgements** This work was funded by BBSRC, BOCM-PAULS and SEERAD.

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# Effects of live weight and condition score on food intake of ewe lambs

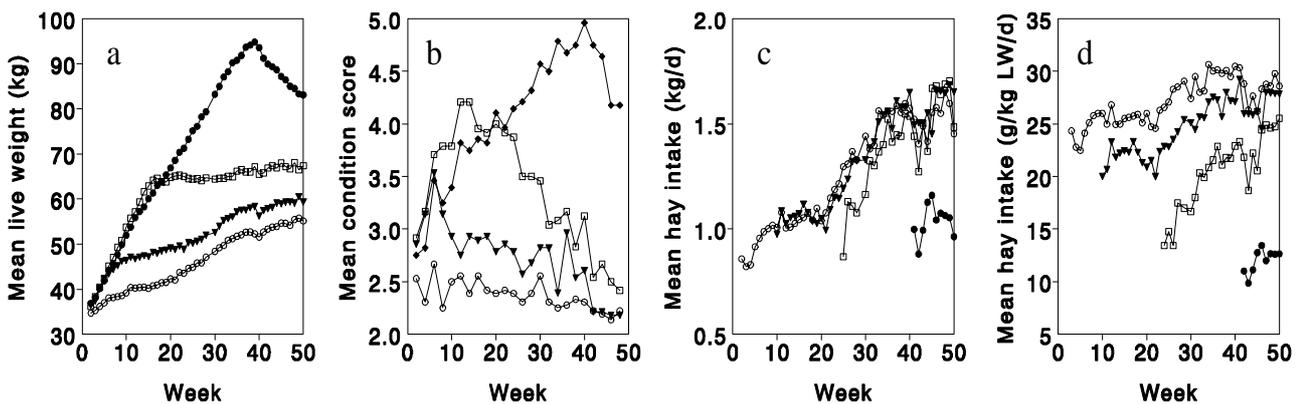
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**Introduction** Food intake (FI) can be predicted on the basis of variables that describe food quality, the environment and the animal. Live weight (LW), in some form or other, is usually the only variable used to describe the animal. Animal fatness, estimated by condition score (CS), can affect intake at a given LW. A simple model to account for that is  $FI = a.LW.(1-b.CS)$  with FI in g/day, LW in kg and CS in units of a scale up to 5. It is evident that food quality will affect parameter  $a$ . Here we test the hypothesis that the parameter  $b$  in this model is significant but not affected by food quality. To that end, we measured LW, CS and intake of three different foods with ewe lambs that showed a large variation in LW and CS as a result of different nutritional histories.

**Materials and methods** Three foods, L (hay, DMD=0.635), M (pellets based on oat feed, DMD=0.627) and H (pellets based on barley, DMD= 0.743) were used in an experiment of 50 weeks on female Greyface\*Texel lambs with a mean initial LW of 35 kg. Ewes consumed more M than H, such that gains in LW and CS were not different. M and H are therefore presented as one food (P) in the first part of the analyses. Six treatments with 8 to 10 lambs each were investigated. Treatment L received hay throughout. Treatments P45L, P65L and P95L received P from week 1 but were switched to hay after reaching 45, 65 or 95 kg LW, respectively. Two further groups received hay from week 1 but were switched to M or H after reaching 45 kg LW. FI and LW were recorded weekly and CS biweekly. Before fitting the model, CS was smoothed and values for missing weeks were interpolated. ANOVA was used to test for effects of food type on the parameters of the model fitted to all ewe/food-type combinations that showed a range in CS of at least one unit.

**Results** Figure 1 shows the LW, CS and FI of groups L, P45L, P65L and P95L. While consuming hay, all treatments lost CS but whereas the LW of groups L and P45L increased throughout, that of P65L was almost static and that of P95L decreased. Fat ewes with a LW of 90 kg consumed less hay per day than lean animals with a lower live weight.



**Figure 1.** Mean LW (a), CS (b), hay intake (c) and hay intake/kg LW (d) of ewes in treatments L (hay trough-out, o), P45L (pellets to 45 kg, then hay, ▼), P65L (pellets to 65 kg, then hay, □) and P95L (pellets to 95 kg, then hay, ●).

A comparison of Figs 1d and 1b shows that CS and hay intake per kg LW was negatively correlated, between and within treatments. Similarly, animals in treatments L45M and L45H had lower CS and consumed more P at similar LW than animals that received P from week 1 (these data are not shown here). The differences between treatments disappeared when CS became similar. The model  $FI = a.LW.(1-b.CS)$  was fitted to 71 ewe/food-type combinations ( $n=16, 30$  and  $25$  for L, M and H, respectively). Parameter  $a$  was affected ( $P < 0.0001$ ) by food type (mean  $\pm$  se  $39 \pm 2.2, 89 \pm 3.0$  and  $80 \pm 3.6$  for L, M and H) but parameter  $b$  was not ( $P = 0.73$ ; mean  $0.147, 0.140$  and  $0.144$  for L, M and H; overall mean  $0.143, se 0.004$ ).

**Conclusions** Many models predict an increase in FI with increasing LW in sheep. Our data show that FI per unit LW is strongly affected by animal fatness as estimated from CS, the latter frequently being the single other animal characteristic that is available. Food quality had a large effect on FI per kg LW (parameter  $a$ ), as expected for foods of such different qualities. However, the parameter related to CS ( $b$ ) was not affected by food type. This suggests that a change of one unit CS has a proportional effect on FI per kg LW independent of food type. As only three foods were included in the present analysis, this finding needs further confirmation. Experimental animals were all ewes of the same crossbreed but sex and genotype may well affect parameter values. Additional relevant data are being collected.

**Acknowledgements** We gratefully acknowledge the assistance of David Anderson and Terry McHale with collection of the data. The work was funded by SEERAD.

## Pressed sugar beet pulp as an alternative fibre source for lactating dairy cows

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**Introduction** A significant proportion of the grass silage fed to lactating dairy cows may be of only modest quality due either to delayed harvesting and/or poor ensiling conditions. In such situations, both total feed intake and milk production are likely to be compromised with the consequent need to feed more concentrates. Part of this effect is considered to be due to the development of a solid mass of digesta in the rumen, with loss of the normal layered or biphasic stratification of rumen contents. Under such conditions, rumen motility, rate of forage digestion and hence voluntary feed intake will be compromised. Mertens (1997) stressed that chemical definition of dietary fibre such as neutral- (NDF) or acid-detergent (ADF) fibre content was an inadequate description of the fibre content of a diet as it affects rumen function and animal performance. Consequently he proposed both effective NDF (eNDF; ability of a feed to replace a roughage with no negative effect on milk fat content) and physically effective NDF (peNDF; a measure of the physical properties of fibre as it stimulates chewing activity and development of the biphasic stratification of rumen contents) as additional descriptors of the physical characteristics of dietary fibre but to date these concepts have attracted limited attention in the UK. This study examined the effect of replacing increasing amounts of grass silage (GS) on a dry matter (DM) basis in a silage:concentrate ration with pressed sugar beet pulp (PP) on various processes of digestion in the rumen of lactating dairy cows, specifically in relation to chewing activity and rumen mat density.

**Materials and methods** Four multiparous Holstein-Friesian cows in late lactation were used. These had previously been fitted with permanent cannulae in the dorsal sac of the rumen. They were held in tie stalls and randomly allocated to a 4x4 Latin square with each period being of 4 weeks duration. The control ration comprised of GS and concentrates (60:40 DM basis) and compared with 3 treatments based on 10, 20 and 30% PP (PP1, PP2 and PP3 respectively) as direct replacements for GS. The GS had a DM content of 216g/kg fresh weight with 549g NDF, 101g crude protein (CP) and 10.2MJ metabolisable energy (ME)/kg DM. PP, which was clamped prior to feeding, had a DM content of 230g/kg fresh weight with 431g NDF, 101g CP and 11.9MJ ME/kgDM. The concentrate contained 254g NDF, 205g CP and 11.7MJ ME/kgDM and was included at 40% of the total ration (DM basis) in all treatments. The GS and PP components of the treatments were mixed fresh daily and fed *ad libitum* along with the concentrate which was top dressed separately onto the 'forage mixture'. All cows were fed at 0830 and 1600h each day and refusals were removed prior to each am feeding. All measurements were taken during the final week of each period and included determination of rumen pH and rumen degradability and rate of passage (chromium mordanted) of a standard grass silage using previously described techniques. Chewing activity was determined by visual observation of the chewing time and number of chews spent on each bolus on 4 separate occasions with 4 boli being observed at each time (16 boli per cow per period). Rumen mat density was determined by inserting a prototype probe on 3 separate occasions into the rumen contents via the permanent cannula in a direction towards the rumen floor and in a forward direction towards the reticulum. Assessment of the resistance of the rumen digesta was then obtained as the probe was passed through the rumen mat by means of a pressure gauge attached to the probe.

**Results** Replacing increasing amounts of GS with PP caused marked improvements in total DM intake with a significant ( $P<0.05$ ) increase at the highest level of inclusion (control, 13.8; PP3, 19.4 kg/d;  $p<0.05$ ), associated with increases in NDF and ADF intake of 29 and 14% respectively. Rumen pH was largely unaffected by treatment between the am and pm feed but a noticeable reduction occurred on all PP containing treatments between 4 and 6 hrs post pm feeding, with minimal values of 5.7 to 5.8 compared with 6.2 for the control. Neither effective DM degradability of GS nor rate of passage of mordanted GS were significantly affected although a non significant decline in rate of degradation was observed as PP inclusion levels increased. In contrast chewing activity was significantly ( $P<0.05$ ) affected by inclusion of PP in the ration with both increased bolus chewing time (47.1 v 50.3 secs/bolus) and chews per bolus (49.6 v 54.5), although some of these effects may have been mediated through the increased DM intake noted on the PP treatments. In addition, a marked reduction in rumen mat density (control, 51.1; PP3, 45.3 bars) was observed and whilst this did not achieve statistical significance its importance should not be underestimated when the higher DM intakes on the PP treatments are taken into account.

**Conclusions** When grass silage of modest quality, but typical of many silages fed on UK dairy farms, was sequentially replaced with PP, marked improvements in DM intake (+41%) were noted, associated with significant changes in both chewing activity and rumen mat density. It is concluded that part replacement of GS with PP provided an improved rumen environment more conducive to the efficient utilisation of ingested fibre. These results add support to the contention that dietary fibre must be assessed in both physical and chemical terms. They also indicate a highly beneficial role for pressed sugar beet pulp in the ration of lactating dairy cows when grass silage quality is modest and likely to limit both feed intake and lactational performance.

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# The effect of grass and maize silage quality on diet digestibility and performance of beef cattle

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**Introduction** A preliminary study at this Institute indicated that inclusion of high quality maize silage in a grass silage-based diet could promote higher forage intakes in beef cattle, but the response to inclusion of maize silage was affected by the quality of grass silage. The objective of this study was to further examine the effects of grass (GS) and maize (MS) silage qualities on intake characteristics, and to evaluate the influence of forage offered on animal performance.

**Materials and methods** 72 continental cross beef cattle, mean initial live weight 485 (sd 24.0) kg, were blocked for live weight and allocated to one of 6 dietary treatments in a continuous design, randomised block experiment. Treatments comprised each of two GS's offered as the sole forage, or each GS offered in a 60:40 mixture (DM basis) with one of two MS's. All diets were supplemented with 3kg/head/day concentrates throughout the 96-day trial period, and were offered *ad libitum*, once daily, through individual Calan gates. The concentrate supplement was formulated such that the crude protein (CP) content of the diet with the lowest CP content was as close as possible to 140g/kg DM. Predicted concentrate starch content was 352 g/kg DM. The mean DM (g/kg), NH<sub>3</sub>-N (proportion of total N) and pH values of the two GS's offered were 231 and 277; 0.10 and 0.08; and 3.95 and 3.91, for GS 1 and GS 2 respectively. The mean DM (g/kg) and predicted starch (g/kg DM) contents of the two MS's were 234 and 90, and 372 and 350, for MS 1 and MS 2 respectively. Total diet digestibilities were determined in a 4-period, partially-balanced, changeover design experiment, with 6 additional animals. Animals on the main study were weighed weekly and liveweight gain determined by regression. Balance data were analysed using the REML technique in Genstat 5, while other data were subjected to ANOVA with forage type (GS or MS) as the main factor.

**Results** Digestibility and animal performance data are presented in Table 1. Predicted total diet metabolisable energy (ME) concentration was lowest with diets containing GS 1 as the sole forage and highest where GS 2 was offered as the sole forage ( $P < 0.05$ ), however type of MS included in the forage mixture had no significant influence on this parameter ( $P > 0.05$ ). DM digestibility was highest with diets containing GS 2 (mean 0.775) compared with those containing GS 1 (mean 0.733) ( $P < 0.001$ ). Total diet DM and ME intakes were highest with diets containing a mixture of GS 2 and MS 2, and lowest where GS 1 was offered as the sole forage ( $P < 0.05$ ). Furthermore, incorporating MS into GS diets increased intakes (numerically, and in most cases statistically) in all treatments. Final animal live weights and liveweight gains were greater with diets containing GS 2 (means of 599 kg and 1187 g/d respectively), compared to those containing GS 1 (means of 574 kg and 1050 g/d respectively) (at least  $P < 0.05$ ), while mean values for both parameters were similar with and without MS inclusion ( $P > 0.05$ ). Carcass weights and rates of carcass gain were greatest with diets containing GS 2 (means of 327 kg and 857 g/d respectively), compared to those containing GS 1 (means of 310 kg and 699 g/d respectively) ( $P < 0.001$ ). Despite variation in means across individual treatments, feed conversion efficiency for carcass gains (DM or ME basis) was poorer with diets containing GS 1 compared to those containing GS 2 ( $P < 0.05$  for DM), and was poorer where MS 2 was included in the diet compared with diets containing no MS ( $P < 0.05$  for DM). Forage type offered had no influence on either killing out proportion or carcass conformation grade ( $P > 0.05$ ). Carcass fat was higher where GS 2 was included in the diet (either as the sole forage or as a component of the total forage) ( $P < 0.01$ ), while inclusion of MS did not influence this parameter ( $P > 0.05$ ).

**Table 1** Total diet digestibility and animal performance results

Forage offered	GS 1	GS 2	GS 1 MS 1	GS 1 MS 2	GS 2 MS 1	GS 2 MS 2	sed	GS	MS	GS*MS
<b>Balance</b>										
ME (MJ/kg DM)	11.51	12.23	11.70	11.76	12.12	11.95	0.267	*	NS	*
DM digestibility	0.727	0.781	0.732	0.742	0.769	0.775	0.0072	***	NS	***
<b>Animal intake and performance</b>										
Total DMI (kg/d)	7.40	8.68	8.53	8.23	8.90	9.62	0.262	***	***	*
Total MEI (MJ/d)	85.1	106.2	99.8	96.8	107.9	115.0	3.11	***	***	*
Final live weight (kg)	569.7	595.5	580.1	573.1	601.7	600.2	9.40	***	NS	NS
Liveweight gain (g/d)	981	1166	1134	1037	1247	1147	92.3	*	NS	NS
Carcass weight (kg)	310.3	324.6	311.8	308.2	328.7	327.0	4.82	***	NS	NS
Carcass gain (g/d)	699	808	715	683	907	858	51.3	***	NS	NS
DMI/carcass gain (kg/kg)	11.11	10.51	12.28	12.14	9.87	12.32	0.807	*	*	NS
Carcass gain/MEI (g/MJ)	8.15	7.93	7.13	6.96	8.29	7.36	0.486	NS	NS	NS
Kill out (g/kg)	546	546	532	534	544	539	6.8	NS	NS	NS
Carcass conformation <sup>1</sup>	2.8	2.6	2.5	2.5	2.7	2.6	0.14	NS	NS	NS
Carcass fat class <sup>2</sup>	2.5	3.4	2.9	3.1	3.2	3.2	0.23	**	NS	*

<sup>1</sup> EUROP scale : 5, 4, 3, 2, 1 respectively; <sup>2</sup> EU fat classification where 5 is the highest fat cover

**Conclusions** The results indicate that inclusion of MS in GS-based diets increased total DM and ME intakes compared to GS offered as the sole forage. However, these higher intakes were not translated into improved animal performance (live weight or carcass gains), such that feed efficiency generally decreased with inclusion of MS in the diet.

# Intake, growth and feed conversion in weaned suckled bulls finished on a cereal-based ration

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**Introduction** Enhanced economic sustainability may be achievable by upland beef farmers if they could finish weaned suckled calves on-farm using purchased feeds, rather than selling store animals. The objectives of this study were to examine voluntary feed intake, growth rate and carcass slaughter parameters of weaned bulls from an upland suckler herd finished intensively from approximately 9 months of age using a cereal-based ration.

**Materials and methods** A 2 x 2 factorial continuous design experiment was conducted to determine voluntary dry matter intake (DMI), liveweight gain (LWG), feed conversion ratio (FCR) and carcass characteristics in suckled bulls weaned at approximately 8 months of age. Experimental factors were sire breed (S) and dam breed (D). Aberdeen Angus (AA) or Charolais (CH) were used as sire breeds on either Belgian Blue x Holstein (BB) or Simmental x Holstein (SIM) cows as dam breeds. A total of 36 bulls were used (12 pens) with 3 bulls/pen and 3 pen replicates of the AA/BB, AA/SIM, CH/BB and CH/SIM breed groups. Following a 5 week pre-trial period when weaned bulls were gradually introduced to the trial ration, all animals were offered a cereal-based diet *ad libitum* (DM: 866; ME: 12.3; CP 186) from weeks 1-10 of the trial and a lower protein cereal based diet (DM: 850; ME: 12.6; CP: 154) from week 10 until slaughter. These diets contained rolled barley, rapeseed meal, molasses, minerals and molassed sugar beet pulp as a fibre source. DMI was determined for each pen on a weekly basis and individual bull LWG determined by linear regression on weekly liveweight (LW) measurements. For each of the four bull breed groups, the rate of change in LWG as bulls grew to heavier weights was assessed by linear regression of weekly LWG against average weekly LW. After selection for slaughter at a target condition of R4L, cold carcass weight (CCW), killing out proportion (KO) and fatness and conformation scores on a 15-point scale were derived from carcass gradings for each bull. Analysis of variance for DMI and FCR were carried out on a pen basis and for LWG and carcass data on an individual bull basis.

**Results** Average DMI, LWG, FCR (kg DMI/kg LWG), days of age at slaughter (AGE), final LW (FLW), CCW, KO and carcass scores are given in Table 1 along with the F-test significance of the main S and D effects. Daily DMI and fat scores were similar between breed groups. CH sired bulls had higher LWG's (P<0.001), finished at younger AGE (P<0.01) with better FCR (P<0.01), superior KO proportion (P<0.05) and produced heavier carcasses (P<0.001) with better conformation (P<0.001) than AA sired bulls. Bulls from SIM dams had higher LWG's (P<0.01) and produced heavier carcasses (P<0.05) than bulls from BB dams. Linear regression equations comparing the rate of LWG with average bull LW for each of the breed combinations are given in Table 2. Regression co-efficients (b) for each breed group indicated only minor changes in the rate of LWG as bull LW increased throughout the trial. After including £183/bull headage-based subsidy payments and no market price premium for the AA bulls, Gross Margin figures (£/bull) were 127, 139, 174 and 198 for the AA/BB, AA/SIM, CH/BB and CH/SIM bulls respectively.

**Table 1.** Average intake, liveweight gain, feed conversion and carcass characteristics in weaned suckled bulls.

	Breed combination				sed	Sig <sup>s</sup>			R <sup>2</sup>
	AA/BB	AA/SIM	CH/BB	CH/SIM		S	D		
DMI (kg/d)	10.6	10.6	11.0	10.9	0.46			AA/BB	
(g/kg LW)	22.4	21.8	21.2	20.2	1.24				2.21 -0.0004 0.0005
(g/kg LW <sup>0.75</sup> )	104	102	101	97	5.48				
LWG (kg/d)	2.07 <sup>a</sup>	2.11 <sup>a</sup>	2.34 <sup>b</sup>	2.65 <sup>c</sup>	0.08	***	**	AA/SIM	
FCR	5.14 <sup>a</sup>	5.06 <sup>a</sup>	4.70 <sup>a</sup>	4.12 <sup>b</sup>	0.21	**			2.53 -0.0011 0.0035
AGE	387 <sup>a</sup>	381 <sup>ab</sup>	374 <sup>bc</sup>	366 <sup>c</sup>	5.4	**		CH/BB	
FLW (kg)	565 <sup>a</sup>	581 <sup>a</sup>	620 <sup>b</sup>	645 <sup>c</sup>	9.6	***	**		1.61 0.0015 0.0075
CCW (kg)	309 <sup>a</sup>	318 <sup>a</sup>	348 <sup>b</sup>	365 <sup>c</sup>	6.6	***	*		
KO (g/kg)	548 <sup>a</sup>	548 <sup>a</sup>	561 <sup>b</sup>	565 <sup>b</sup>	9.4	*		CH/SIM	
Fat score	7.5	7.3	6.8	6.8	0.57				2.39 0.0002 0.0002
Conf score	9.3 <sup>a</sup>	10.3 <sup>a</sup>	12.4 <sup>b</sup>	12.8 <sup>b</sup>	0.97	***			

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

<sup>s</sup>. SxD F-test was significant for LWG (P<0.05).

**Table 2.** Linear regression equations comparing the rate of bull LWG against bull LW (y = a + bx)

Bull Breed	a	b	R <sup>2</sup>
AA/BB			
	2.21	-0.0004	0.0005
AA/SIM			
	2.53	-0.0011	0.0035
CH/BB			
	1.61	0.0015	0.0075
CH/SIM			
	2.39	0.0002	0.0002

**Conclusions** Excellent physical performance can be achieved from 3/4 beef-bred suckled bulls weaned at 8 months old and finished on-farm using purchased cereal-based rations. CH sired bulls out-performed AA sired bulls, especially when produced from SIM cows. There was no evidence to indicate that the rate of LWG changes as bulls grow from 9 months of age until slaughter at 12 to 13 months.

**Acknowledgements** This work was funded by DEFRA, MLC, Dovecote Park and Waitrose Ltd with further support from the Aberdeen Angus Cattle Society and the British Belgian Blue Cattle Society.

## Preliminary investigations of behavioural and physiological responses to castration in horses

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**Introduction** Behavioural responses to pain are highly species specific and reflect varying strategies for survival. As prey animals, horses may fail to show obvious pain responses, instead masking pain to reduce predation through selection as the weakest of a group (Anil *et al.*, 2002). Price *et al.* (2002) identified disagreement amongst vets regarding pain assessment and management in horses. This was highlighted by recent debate concerning the existence of post-castration pain and the necessity for analgesia in equines (e.g., Capner 2001; Green 2001). While optimal assessment and management of pain is an important equine welfare issue, the behaviours of horses in response to pain are poorly defined (Raekallio *et al.* 1997) and the relevance of physiological indicators not confirmed. Palpation or human interaction tests, used in other species (e.g. Holton *et al.* 1998), have yet to be validated in horses. This study aimed to identify and quantify potential behavioural indicators of post-castration pain in horses.

**Materials and Methods** Group 1 (n=12) were thoroughbred horses (age 2-5yrs) were castrated using a closed technique under standing sedation. Group 2 (n=5) were male thoroughbred horses (age 2-5 yrs) non-surgical 'pain-free' controls. Sedation protocols varied dependant on veterinary preference and horse temperament. Horses number 1-10 received detomidine (10µg/kg) combined with butorphanol (20µg/kg). Horse number 11 received ACP (0.075µg/kg) and horse 12 received ACP (0.025mg/kg) detomidine (10µg/kg) and butorphanol (20µg/kg). Local anaesthesia was administered with 2% lignocaine. Horses received phenylbutazone (4.4mg/kg) pre and 1 gram bid for 5-7 days post-operatively. Surgery horses were assessed pre-operatively (baseline) and at 6, 24 and 48 hours post-operatively. Group 2 horses were assessed at time 0 (baseline) and 6, 24 and 48 hours later. Behavioural assessment included 1) observation of undisturbed behaviour in the stable over a 30-minute period; 2) responses to a standardized palpation test, where a handler approached the horse, placed their hand midway down the neck and proceeded to run the hand (applying a constant pressure) down towards the girth (interactive behaviour). In both measures, duration of behaviours, such as locomotion, feeding and postures, were recorded using point sampling. Frequency of behavioural events such as tail flicking and leg movements was recorded continuously. Heart rate was recorded during both undisturbed and interactive behavioural sampling via telemetry. Respiration rate was recorded by direct observation immediately after each behavioural sample. Analysis involved within-group comparisons of post-operative and baseline data (each individual acting as its own control), using two-way ANOVA or Friedman tests where appropriate. Post-hoc paired t test or Wilcoxon signed rank tests were applied to significant results. 'Control' and 'surgery' groups were compared at each individual time point, using a two-sample t test or Mann-Whitney U test.

**Results** During undisturbed sampling 'within-group' analysis of 'surgery' horses showed increased resting at 6 hours post-castration (P<0.05), accompanied by an increase in 'head level with withers' posture (P<0.05). 'Within-group' trends for increased stamping (P=0.063) and decreased grooming (P = 0.052) were seen post-operatively in surgery horses. Interactive sampling found increased time spent with ears back at 6 and 24 hours (significant at 24 P<0.05) in post-castration horses. These changes were not seen in 'control' groups. Comparisons between 'control' and 'surgery' groups found castrated horses to perform significantly more stamping at 6 hours post-operatively than control horses (P<0.05). No other differences were identified. No changes were found in physiological variables over time or between groups.

**Conclusions** The behavioural changes observed may be indicative of pain or discomfort. Post-operative behavioural changes are unlikely to be associated with sedative 'hangover' due to the short half-lives of the drugs used (Dyke 1993; Plumb 1999). The results suggest that changes in behaviour could be used as indicators of equine pain, however, basic non-invasive physiological variables are not reliable indicators of pain. More work is needed to develop and validate reliable methods of pain assessment in horses.

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# Factors influencing stereotypical behaviour patterns in horses: a review of 52 clinical cases

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**Introduction** Behaviours such as weaving, box-walking and wind-sucking have traditionally been regarded as undesirable behaviours or 'vices' by horse owners, which has led to 'treatment' regimes that aimed to physically prevent the performance of the behaviour rather than understand the underlying causes for it. In recent years, however, a number of studies have shed light on the epidemiology of these behaviours (e.g. Luescher et al 1998), leading to the development of more welfare compatible treatment options. In this study, a clinical population of horses presented with stereotypical behaviours is examined for relationships between presenting signs and historical and observational findings.

**Materials and Methods** The data for this study was collected from the case records of 52 clinical cases of equine stereotypies referred to a veterinary behaviour specialist by first opinion veterinary surgeons in the South of England between the years 1992 and 1998. Each horse was visited in its home environment for a behaviour consultation of between two and four hours, prior to which each owner was sent a questionnaire which included details of the specific problem behaviour, the signalment of the horse, the management system under which it was kept, and any information that was available about the history of the horse. During the consultation the horse was observed displaying the stereotypical behaviour where possible, or further information was obtained from owners as to the location, timing, and specific triggers for the problem behaviour. The following variables were extracted from the case questionnaires and reports: type of horse; age; sex; colour; purpose for which the horse was used; type of stereotypy; age of onset of stereotypy; and triggering stimulus for stereotypical behaviour to occur. The population consisted of 19 Thoroughbreds (TB) 7 'Warmbloods', 6 'Coldbloods' 16 TB first crosses, and 4 Arabs or Anglo-Arabs. 26 of the horses were geldings, 5 entire males and 21 were mares, and the age range for the population was 4 to 17 years. 24 of the horses were kept as general riding horses, 10 were used exclusively for dressage, 9 were used for eventing and / or show-jumping, and 7 for racing or point-to-pointing. The relationship between the age of horses and other variables was examined using Kruskal-Wallis tests. All other variables were compared using chi-square tests.

**Results** Of the 52 horses, 17 were weaving, 9 were crib-biters, 3 were windsucking, 9 were box-walking, 7 were head nodding, 5 were displaying stereotypical lip or tongue movements (including wall or door licking), and 2 were self mutilating. The self mutilating horses were excluded from further analysis because of the small number, and windsucking and crib-biting horses were combined into a single category. No significant relationship was found between type of stereotypy and sex, type of horse, purpose of horse, or colour. A significant relationship was found between type of stereotypy and age of onset ( $\chi^2 = 38.142$ ,  $df = 12$ ,  $p < 0.01$ ), with a higher than expected count (40%) of lip and tongue movement stereotypies starting between 1 and 3 years of age, and 50% of crib-biting and windsucking starting at less than 1 year of age. Unfortunately, in the majority of cases ( $n = 37$ ), age of onset of the behaviour was unknown due to change of ownership. Age of onset was also related to the purpose for which the horse was kept, as those used for racing / point-to-point were more likely to have an age of onset less than 1 year of age, and less likely to have an unknown age of onset than those used for other purposes ( $df = 9$ ,  $p = 0.01$ ). This result needs to be viewed with some caution, however, as the age of the horse at the time of the consultation may be a confounding factor. Horses in the racing group were significantly younger at the time of consultation, and were hence less likely to have changed hands than the older horses in the other groups. In fact, age at time of consultation was found to be significantly related to age of onset ( $df = 3$ ,  $p < 0.01$ ). A significant relationship was found, however, between presenting stereotypy and the factor identified as the primary trigger for the behaviour to occur ( $\chi^2 = 41.170$ ,  $df = 8$ ,  $p < 0.01$ ). Stimuli identified as those responsible for triggering the stereotypy for each horse were simplified into 3 categories, which were: (i) Anticipatory; behaviour occurs with human activities causing the horse excitement, anticipation or frustration, such as preparing feeds, preparing to turn a horse out, tacking up for exercise, (ii) No stimuli; behaviour occurs in the apparent absence of triggering stimuli in the stable or whilst at grass, and (iii) Conspecific; behaviour occurs in response to activity of other horses, such as other horses passing the stable, or calling from out of visual contact. A higher than expected count of weaving horses (71%) were primarily triggered to show this behaviour with anticipatory triggers. Similarly, 92% of windsuckers did so with no apparent stimulus, and 56% of box-walkers displayed this behaviour with conspecific activity. Precipitating factor was not related to type, age, sex or purpose.

**Conclusion** The results of this study suggest that the age of onset and triggering factors for stereotypical behaviour in horses vary with physical presentation. The earlier age of onset for crib-biting and wind-sucking than for other stereotypies is consistent with the findings of Waters et al. (2002) in their longitudinal study into the effect of weaning. In addition, the relationship of stereotypy type and triggering factor supports the findings of other authors, such as Cooper et al (2000) in the hypothesis that the origin of these behaviours are not uniform, and involve different motivational states, stages of development and possibly different neuroanatomical and neurophysiological changes.

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# The importance of aspects of the cage environment to female laboratory rabbits

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**Introduction** It is generally recommended that female laboratory rabbits are housed socially, although this may not always be possible for experimental reasons. Some newer cage designs allow rabbits in adjacent cages to have visual and minimal tactile contact through a mesh panel. However, it is not known whether rabbits find such limited contact rewarding. It is becoming increasingly common for rabbit cages to be fitted with a platform, which provides a raised area as well as a darkened and often enclosed area underneath, which can act as a bolt-hole. Rabbits may be able to see into adjacent cages from on top of platforms, and it is not known whether they use the platform primarily to rest on or as a means of gaining social contact, or both. The aim of this experiment was to investigate the motivation of rabbits for visual and minimal tactile contact with a conspecific and a raised platform/bolt-hole.

**Materials and methods** Eleven female New Zealand White rabbits (22-27 weeks old) were housed in plus (+) shaped sets of apparatus for the duration of the experiment. One of the four arms of the apparatus (resource cages) contained a platform and another allowed the rabbits to gain visual and minimal tactile contact with a conspecific through a mesh panel. To allow the relative importance of these resources to be assessed, a high value resource (food) and one which was likely to be of low value (an empty space) were available in the other two resource cages. The cages could only be accessed from the central area of the 'plus' (home cage) via a one-way push-door, with the rabbits exiting via a second one-way push-door. Water was provided in the home cage. Following a familiarisation period of free access to resource cages, a 250gram weight was attached to each access door (exit doors were always unweighted) and was increased by 250grams every two days. This was continued until the rabbits had not pushed into the food cage for 20 hours, after which the trial was ended (for welfare reasons). The number and mean duration of visits and total time spent in each cage at each weight were calculated. The effect of weight on resource use was analysed using General Linear Models. Two economic measures were used to assess the relative importance of the resources: the maximum price paid (i.e. maximum weight pushed through) and the total expenditure per day (cumulative weight pushed through). Comparisons between resources were made using Friedman two-way analysis of variance with paired comparisons. The time spent interacting with the resource itself while in the cage was also analysed for the platform and social contact cages.

**Results** There was a difference in the maximum price paid for the resources (Table 1;  $S=15.35$ ,  $p<0.05$ ), with heavier weights pushed through for food, social contact and a platform than for the empty cage ( $z=19.5$ ,  $11.0$  and  $13.5$  respectively, all  $p<0.05$ ). A difference was also found in total expenditure per day ( $S=24.27$ ,  $p<0.05$ ), with expenditure for food being greater than that for the platform and the empty cage ( $z=17$  and  $26$ , both  $p<0.05$ ), as was expenditure for social contact ( $z=14$  and  $23$ , both  $p<0.05$ ). Overall, more visits were made to the food and social contact cages than the platform and empty cages ( $F_{(3,186)}=36.08$ ,  $p<0.001$ ; all  $p<0.001$ ). Most time was spent in the home cage (39%,  $sd \pm 19$ ), followed by the platform cage (27%,  $sd \pm 15$ ), social contact cage (17%,  $sd \pm 14$ ), food cage (14%,  $sd \pm 6$ ) and empty cage (4%,  $sd \pm 4$ ). The rabbits altered their daily time budget as the weight on the push-doors increased, with fewer visits made to each resource cage ( $F_{(1,186)}=161.96$ ,  $p<0.001$ ), but with an increase in mean visit duration ( $F_{(1,241)}=81.06$ ,  $p<0.001$ ). The effect of the weight on the total duration of time spent in the cages varied, with the time in the home cage increasing, and the time in the platform cage decreasing. Analysis of resource use in the social contact cage found that the rabbits spent over a third of the time out of direct visual contact with the other rabbit. In the platform cage, the rabbits spent approximately 95% of their time lying in front of the platform, rather than on or under it.

**Table 1** Medians of the maximum price and total expenditure paid for resources

	Food	Social contact	Platform	Empty cage
Maximum price paid	1000	1000	1000	1000
(Q1, Q3)	(750, 1250)	(750, 1250)	(750, 1250)	(750, 1250)
Total expenditure per day (Q1, Q3)	8700	3900	6958	1083
	(8000, 11500)	(3250, 5500)	(4750, 10600)	(350, 2417)

**Conclusions** Food and social contact were of equal and most importance to the rabbits with both economic measures used. The importance of the platform differed according to the measure used, with rabbits being as motivated as for food and social contact in terms of the maximum price paid, but less motivated according to the total expenditure per day. The fact that the rabbits pushed into the social contact cage, but then spent less than two thirds of their time in visual contact suggests that proximity to another rabbit, and/or olfactory contact are of value to rabbits. The findings also suggest that the feature of the platform that was of most value was proximity to the platform, which was likely to be due to the bolt-hole underneath the platform providing a means of escape if the rabbit feels threatened. It is recommended that singly housed female laboratory rabbits should have visual and minimal tactile contact with conspecifics, and that they should also be able to avoid such contact. It is also recommended that cages should be fitted with a platform/bolt-hole.

## The effects of games on the dog-owner relationship

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**Introduction** It is often claimed that certain dominance-related problems in dogs can be triggered by the games played by dog and owner. In particular it is thought that allowing a dog to win uncontrolled games such as Tug-of-War will increase the likelihood of it attempting to become dominant over its owner. However questionnaires (Goodloe & Borchelt, 1998) and experimental studies of Labrador and Golden Retrievers (Rooney & Bradshaw, 2002) have found no evidence for these postulated effects. In this paper we further investigate possible links between the types of games played in the domestic environment and both dominance and attachment dimensions of the dog-owner relationship.

**Methods** Fifty dog-owner partnerships were recruited. The dogs were aged between 20 months and 14 years (mean =7 years); there were 29 males and 21 females representing 17 different breeds. When classified according to Kennel Club categories there were 17 gundogs, 16 working dogs, 10 terriers, 3 hounds, 2 toy and 2 utility dogs. Each dog and owner was filmed during a three-minute play session in which the owner chose the games played. All partnerships then undertook a one-hour test designed to measure dominance- and attachment-related behaviour of the dog. This test included sixteen components during which the owner performed a variety of actions towards the dog including removing its food bowl, grooming it and leaving it alone for three minutes. The test was video recorded throughout and from the tapes, the dog's behaviour recorded in 88 variables, which were reduced by Principal Components Analysis to two dominance-related factors (Amenability and Confident Interactivity) and four factors describing aspects of attachment (Non-Specific Attention-Seeking, Preference for Owner, Preference for Unfamiliar Person, and Separation-Related Behaviour). The effects of the types and style of games played on these six factors were tested using nonparametric statistical tests (Spearman Rank Correlation, Kruskal-Wallis, Mann Whitney U, and Fisher's Exact tests).

**Results** Dogs which played Rough-and-Tumble scored higher for Amenability ( $U=207$ ,  $p<0.05$ ), and lower on Separation-Related Behaviour ( $U=210$ ,  $p=0.05$ ), than dogs which played other types of games. Dogs which played Tug-of-War ( $U=197$ ,  $p<0.05$ ) and also those which played Fetch ( $U=192$ ,  $p<0.05$ ), scored high on Confident Interactivity, but whether they tended to win or lose these games had no consistent effect on any of their test scores. If the dog rather than the owner started the majority of the games, the dog was significantly less Amenable ( $U=162$ ,  $p=0.005$ ) and more likely to exhibit aggression (Fisher's exact test:  $p<0.01$ ).

**Conclusions** The test procedure proved to be an effective way of quantifying both attachment and dominance dimensions of a dog-owner relationship. The results of this study provide no evidence that the outcome of games have a significant effect upon dominance dimensions of dog-human relationships, but suggest that attachment dimensions may be affected by playing games which involve considerable body contact. We conclude that the way in which dogs play reflects general attributes of their temperament and their relationship with their owner, but for the majority of dogs the outcome of games are not deterministic. However, we suggest that if play signals are absent or misinterpreted then games may have more serious consequences (Rooney *et al.* 2001), and for a minority of "potentially dominant" dogs, games may have greater significance. An important aspect of play seemed to be not which player wins the game, but which player initiates it. Dogs which were reported to initiate play frequently scored lower for Amenability and were more likely to exhibit aggression. This is evidence for the popular claim that dogs which are frequently allowed to initiate social interactions also behave with increased dominance towards their owner.

### Acknowledgements

We would like to thank the 50 kind volunteers who were filmed with their dogs. We also thank WALTHAM and the BBSRC for financial support of the project.

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## Dominance hierarchies in domestic cats: useful construct or bad habit?

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**Introduction** In the diagnosis and treatment of behavioural disorders in multi-cat households, it is often assumed that a dominance hierarchy exists between the cats (*e.g.* Crowell-Davis, 2002). While such hierarchies are probably commonplace among dogs, what evidence there is to support the existence of social hierarchies in groups of domestic cats has mainly been gathered from reproductively entire animals, such as single sex laboratory colonies, and free-ranging aggregations of ferals. For example, Natoli *et al.* (2001) used receipt of “submissive” (defensive) behaviour to construct a weakly linear hierarchy in a group of 14 farm cats, but this did not correspond to the hierarchy derived from receipt of affiliative behaviour. We have investigated the alternative hypotheses that apparent dominance hierarchies in multi-cat households may actually be based upon territorial behaviour, or some other undetermined social system.

**Subjects and methods** We observed a group of nine adult cats (3 males, 6 females; age 2-8 years) living in a two-storey house (95m<sup>2</sup> floor area) with free access to the outdoors. All cats had been introduced to the group aged 12 weeks or younger; all were neutered except for the youngest female. Two females, and one male and one female, were same-litter siblings, the other cats were thought to be unrelated. Over a two-week period 112 scans of the position of each cat were taken, and interactions between the cats were recorded during 56 hours of observation; categories of behaviour were aggression (*e.g.* attack, cuff, bite, tail swish while staring), defensive behaviour (*e.g.* flee, ears back, hiss), affiliative behaviour (*e.g.* rub cat, allogroom, miaow) (see Bradshaw 1992 for definitions), and vigilance (watching or staring at another cat, moving to avoid an interaction). Behavioural and positional data were compared by Mann-Whitney U-tests and Spearman rank correlations, which were also used to compare rankings constructed from pairwise net performance of aggression, receipt of defensive behaviour, and receipt of affiliative behaviour. The observations were repeated when the entire female had a litter of kittens, aged 3-5 weeks, in an upstairs bedroom, and performance of behaviour was compared between periods by Wilcoxon matched-pairs signed-ranks tests.

**Results** When there were no kittens, all cats exhibited aggression (106 instances) defensive behaviour (132) affiliative behaviour (143) and vigilance (262). Since approximately one-third of relationships were unresolved in each case, accurate Landau indices could not be calculated, but rankings based on numbers of resolved relationships appeared to be approximately linear for each measure. However, correlations between these rankings were not as expected: aggression ranking was uncorrelated with defensive and affiliative rankings (N=9, rho=0.37, 0.18) but defensive was weakly negatively correlated with affiliative (rho=-0.61, P<0.05). Four of the females preferred to rest in locations that were never occupied by any other cat, and these cats were net recipients of affiliative behaviour (U=0, P<0.05), and net donors of defensive behaviour (U=2.5, P>0.05). Although these observations are consistent with the hypothesis that several of the cats in this group maintained small exclusive territories, there was no correlation between the distance between each cat’s most used resting place and pairwise performance of any of the three classes of behaviour (N=36, rho 0.21 to 0.14); strong negative correlations would be expected if most interactions took place at territory boundaries. In fact, most interactions took place in the area where the food was provided.

When the kittens were present, seven of the adult cats, excluding the mother and one male, restricted themselves to the ground floor of the house, effectively almost doubling the density of cats. These seven did not spend more time out of the house when the kittens were present (median percentage of scans in house; 55% without kittens, 60% with), but they did increase their performance of vigilance by 61% on average (N=7, Z=2.37, P<0.05) and affiliative behaviour by 150% (Z=2.12, P<0.05); aggressive and defensive behaviour increased only slightly (both P>0.5).

**Discussion** Although “hierarchies” could be constructed from three types of social behaviour, these did not correspond, and it is unclear from either this or previous studies which type of behaviour, if any, reflects underlying “dominance”. Both affiliative behaviour (Macdonald *et al.*, 1987), and defensive (“submissive”) behaviour (Natoli *et al.*, 2001) have been suggested as indicators of submission, *i.e.* the inverse of dominance, in the cat; in our study these were negatively correlated, so only one, and conceivably neither, could be an indicator of “dominance”. If dominance was maintained by aggressive or defensive interactions, it would be expected that both would be more frequent when the density of cats was higher; instead, the cats became more vigilant, thereby presumably avoiding agonistic encounters. The higher rate of affiliative behaviour at higher density suggests that this is used to reinforce patterns of coalitions and/or as appeasement.

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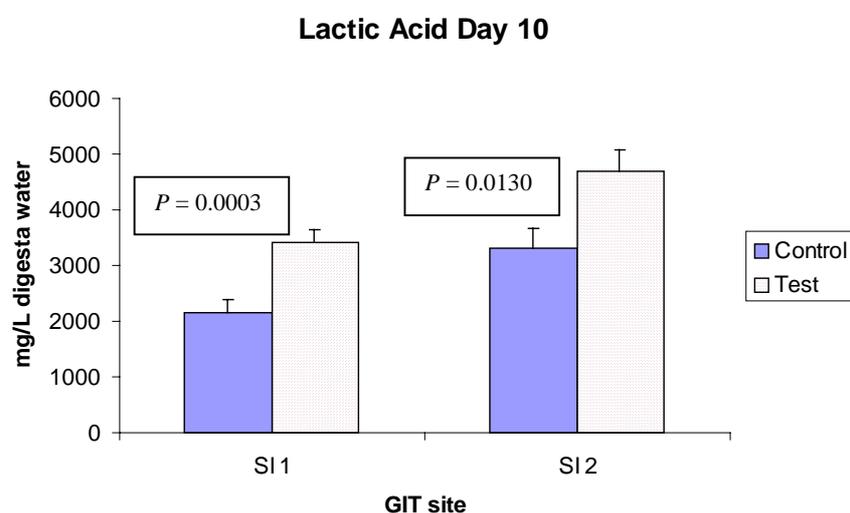
## The effect of dietary fermentable carbohydrates on lactic acid concentration in small intestinal digesta of piglets at ten days after weaning

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**Introduction** It is now accepted that the microbial communities living within the gastro-intestinal tract (GIT) can influence pig health, especially at stressful times such as weaning. Careful design of the animal diet is considered to be an easy way to influence the microflora positively. In this work, fermentable carbohydrates were selected after an *in vitro* test (cumulative gas production- Bosch *et al.*, 2002) for both rate and extent of fermentability. The ingredients were chosen both for their end-products and in terms of their fermentation kinetics so as to stimulate fermentation along the entire GIT. They were incorporated into a test diet (TEST), and compared with a control diet (CONT) which contained minimal fermentable carbohydrates. Digesta were analyzed both in terms of microbial species (molecular techniques) and their end-products (e.g. lactic acid), to determine whether fermentation had, in fact, been stimulated by the TEST diet.

**Materials and methods** Forty-eight piglets were weaned abruptly at 28 days of age, and offered one of two diets. The CONT diet was a semi-purified diet based on maize starch and fishmeal, while the TEST diet had added lactulose (1.0%), inulin (0.75%), wheat starch (5%) and sugarbeet pulp (5%). All piglets were slaughtered on Day 10 after weaning, and digesta collected from the first and second halves of the small intestine (SI1 & SI2). Lactic acid was analyzed in the small intestinal samples and the bacterial composition determined using denaturing gradient gel electrophoresis (DGGE).



**Figure 1** Lactic acid concentration in two halves of the small intestine according to changes in diet.

**Results** Concentrations of lactic acid in SI1 and SI2 on Day 10 after weaning are shown in the graph (Fig.1). Digesta from TEST piglets (n=24) showed significantly higher lactic acid concentrations at both sites (SI1  $P < 0.05$ ; SI2  $P < 0.05$ ) compared with CONT animals (n=24). DGGE analysis showed that Lactobacilli were present as dominant bands for all TEST piglets, while their presence was much more variable for CONT animals. Also, CONT piglets consistently showed the presence of clostridial *spp.*, which were not detected in the TEST animals, showing a clear example of competitive exclusion.

**Conclusions** This work is only part of a large experiment to examine the effect of fermentable carbohydrates on GIT fermentation in weaning piglets, and their effect on animal health. These results indicate that the addition of specific carbohydrates to the diet can lead to increased number and activity of Lactobacilli and have an effect on the presence of clostridial *spp.* Such shifts in the microflora indicate the efficacy of these CHO as prebiotic substances, and may have a potentially positive effect on animal health.

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**Acknowledgements** This project was supported by an EU grant from the HEALTHYPIGUT (QLK5-LT2000-00522).

# Effect of endosperm texture and IBIR rye translocation on performance of piglets between 15-25kg live weight fed diets based on wheat with or without xylanase

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**Introduction** Variability in the nutritive value of wheat is established particularly with young poultry and pigs. However, underlying causes have yet to be established unequivocally. Numerous reports attempting to relate variability to varieties have not proved convincing due to interactions with environmental factors. Furthermore reliance on name alone is not appropriate in attempting to differentiate between varieties as it would give no indication of genetic relationships. One promising approach, is the use of near-isogenic lines, which differ in only one key characteristic whose nutritional significance can thus be examined. This was the basis for establishing the negative effects of the IBIR rye translocation and hard endosperm texture, together with interactions, with poultry (Short et al., 2000). Crucially it was confirmed that investigating the IBIR translocation should not proceed without knowledge of endosperm texture, a requirement overlooked by Lewis et al. (1999). The current programme sought to examine the IBIR translocation and endosperm texture in terms of performance of piglets from 15kg fed diets based on identical formulations but containing wheats of known background; presence of xylanase was the second variable.

**Material and Methods** Seventy-two male hybrid pigs of initial live weight 13.0kg ± 1.2g were individually penned and randomly allocated to one of 12 treatments from 15 to 25 kg live weight. Experiment 1 employed 2 'sister' wheats both of hard endosperm texture but either with (A) or without (B) the IBIR rye translocation. Experiment 2 was based on 4 wheats, 2 of which were with IBIR/Hard (A) and the other 2 without IBIR/Soft (B). Wheat was included at 550g/kg, hipro soya bean meal 345.5g/kg and wheat feed at 70g/kg; amino acids, minerals and a vitamin/mineral premix were added to meet the requirements of the piglet at 15kg. Xylanase (Porzyme 9300) was added, where necessary, at a rate of 1g/kg. Experimental diets were offered *ad libitum* and water was freely available. Feed intake and live weight were recorded weekly. The trial concluded when individual piglets weighed 25kg. Daily live weight gain (DLWG) was calculated as the linear slope of the response of live weight to time (days). Feed intakes (FI) were adjusted accordingly so that the exact amount consumed from 15 to 25kg was calculated; feed conversion ratios (FCR) were then determined.

**Results.** Data for experiment 1 revealed a highly significant effect of wheat type on both DLWG (0.616 for A vs 0.687 for B; sed=0.024, P=0.008) and FCR (respectively; 1.559 vs 1.382 for A and B, sed=0.043 P<0.001). There was no effect of enzyme. The results of Experiment 2 are presented in Tables 1, 2 and 3 respectively for DLWG, FI and FCR. There was no effect of wheat pair type; however, there were significant effects (P=0.006) of xylanase addition for DLWG. There were significant wheat \* xylanase interactions (P=0.015) for both FI and FCR. Enzyme significantly improved FCR for the wheats based on – IBIR/Soft.

**Table 1** Experiment 2; Effect of treatment on DLWG (kg)

Wheat (W)	Xylanase (X)		Mean	ANOVA					
	+	-		W		X		W*X	
- IBIR/S	0.653	0.601	0.627						
+IBIR/H	0.636	0.620	0.628	SEd	P	SEd	P	SEd	P
Mean	0.644	0.611	0.627	0.0117	0.972	0.0117	0.006	0.0165	0.138

**Table 2** Experiment 2; Effect of treatment on FI (kg experimental period)

Wheat (W)	Xylanase (X)		Mean	ANOVA					
	+	-		W		X		W*X	
- IBIR/S	14.1	15.2	14.6						
+IBIR/H	15.0	14.7	14.8	SEd	P	SEd	P	SEd	P
Mean	14.5	15.0	14.8	0.27	0.564	0.27	0.150	0.39	0.015

**Table 3** Experiment 2; Effect of treatment on FCR (kg experimental period)

Wheat (W)	Xylanase (X)		Mean	ANOVA					
	+	-		W		X		W*X	
- IBIR/S	1.412	1.521	1.467						
+IBIR/H	1.497	1.468	1.482	SEd	P	SEd	P	SEd	P
Mean	1.454	1.495	1.48	0.27	0.564	0.27	0.150	0.39	0.015

Data confirm the essential need to characterise wheats more accurately when formulating diets for piglets.

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## The relationship between liveweight and the intake of bulky foods in pigs

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**Introduction** The amount of a bulky food that an animal can eat depends on its capacity for bulk and the bulk content of the food. For pigs between 12 and 40kg the capacity for food bulk was found to be directly proportional to liveweight (Kyriazakis and Emmans, 1995). The way in which the capacity for bulky foods changes with weight above 40 kg is not clear; there is no *a priori* reason to assume that the scaling rule proposed for young pigs will hold in heavier pigs. The applicability of the work in young pigs for use in more mature pigs needs investigation, to develop predictive equations for the whole relevant weight range. An experiment was designed to determine how the capacity for bulk changed with weight; the objective was to develop a relationship between the capacity for food bulk and liveweight.

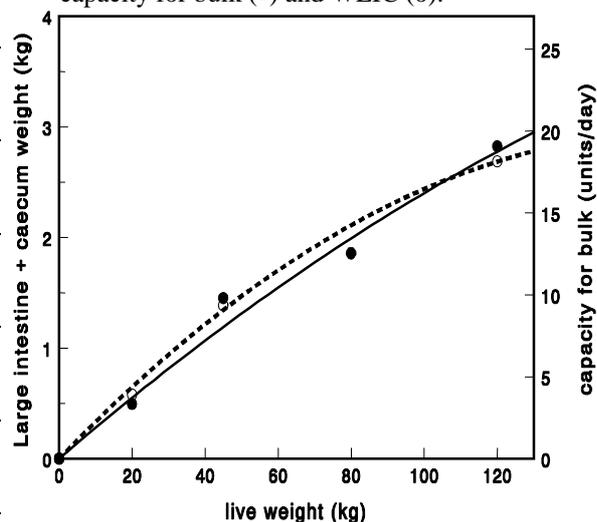
**Materials and methods** Thirty-two commercial hybrid pigs at an average weight of 9.3 (s.d. 1.24) kg were individually penned. Three foods fed *ad libitum* were used. The control food (C), based on micronised wheat, contained 12.9MJ DE and 212g CP per kg of fresh food. The high bulk foods contained either 60% (SBP<sub>60</sub>) or 80% (SBP<sub>80</sub>) unmolassed sugar beet pulp. SBP<sub>60</sub> had 11.3MJ DE and 163g CP, and SBP<sub>80</sub> 10.7MJ DE and 156g CP per kg of fresh food. SBP<sub>60</sub> was fed from 12, 36 or 72kg for a period of 21 days (n=4). SBP<sub>80</sub> was fed from 108kg for 21 days (n=4). For each of these high bulk treatments there was a comparable control treatment used to confirm that the bulky foods used were limiting intake. At the end of each period the pigs were slaughtered and measurements made on the gastrointestinal tract. The data were analysed as a completely randomised design. The variables were: food intake (FI), food intake per kg d (SFI), liveweight gain (LWG), liveweight gain per kg d (SLWG), and the weights of the empty stomach, the small and large intestines, the caecum, the gut fill, and the mesentery. The fixed factor in the model was treatment. To determine the relationship between capacity for bulk and liveweight, intakes (absolute and scaled) were multiplied by the water-holding capacity (WHC) of the food being fed to allow for different foods being used at different liveweights. The capacity for bulk was calculated as FI (kg d<sup>-1</sup>) x WHC (g g food<sup>-1</sup>). Capacity for bulk was determined at different liveweights.

**Results** SBP<sub>60</sub> was limiting at 12kg, but the bulky foods could not be shown to be limiting at 36, 72 or 108kg using LWG (Table 1). However, after allowing for the effects of a change of gut fill, adjusted LWG (aLWG) was less at all weights on the high bulk foods than on C. Although the reductions were significant at 12kg only, the consistency of the reduction strongly indicates that the foods used were limiting and that the data can be used to determine the maximum capacity for bulk at a given weight. Constrained intake was not directly proportional to liveweight beyond 40kg. The capacity for bulk (Cap, kg water holding capacity day<sup>-1</sup>) was related to liveweight (LW, kg) by the quadratic function  $Cap = (0.192.LW) - (0.000299.LW^2)$ . The value of Cap is predicted to reach a maximum when LW = 321 kg. At all weights the high bulk foods caused a significant increase (P < 0.05) in the weights of the stomach, large intestine, caecum and gut fill. Effects on the weight of the small intestine were small. The combined weights of the large intestine and caecum (WLIC) changed with LW in a way that was similar to the way in which Cap changed (Fig.1). In addition the ratio of Cap to WLIC was close to constant.

**Table 1.** Means for intake, LWG, SFI and LWG adjusted for gut fill

LW (kg)	Treatment food	Intake (g day <sup>-1</sup> )	SFI (g kg <sup>-1</sup> day <sup>-1</sup> )	LWG (g day <sup>-1</sup> )	aLWG (g day <sup>-1</sup> )
12	C	927	47.8	743	691
	SBP <sub>60</sub>	1103	56.6	608	503
	s.e.d.	65.8*	3.76*	40.8**	41.6***
36	C	1809	41.4	892	813
	SBP <sub>60</sub>	1695	37.9	760	723
	s.e.d.	125	2.93	74.8	74.8
72	C	2477	31.6	1103	1070
	SBP <sub>60</sub>	2297	29.0	997	889
	s.e.d.	228	2.93	96.0	n.a.
108	C	3095	26.8	1012	958
	SBP <sub>80</sub>	2692	23.1	947	789
	s.e.d.	181	1.55	135.4	129

**Figure 1** Relationships between liveweight and capacity for bulk (●) and WLIC (○).



**Conclusions** Constrained food intake was not directly proportional to liveweight over the whole relevant weight range. A quadratic relationship provided an accurate description of the relationship between capacity for bulk and liveweight. The way in which the weights of the large intestine and caecum changed with liveweight was similar to the way in which capacity for bulk changed with liveweight. This provides some evidence that there is a link between the weight/size of the large intestine and the caecum and capacity for food bulk. The above relationships may be able to be used to develop more accurate intake prediction methods which are relevant across the whole relevant weight range of pigs.

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## Production responses of weaner pigs after chronic exposure to airborne dust and ammonia

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**Introduction** Housed pigs are exposed chronically to aerial pollutants, principally dust and ammonia, at concentrations that may affect performance, possibly by raising the incidence and prevalence of multi-factorial respiratory diseases. Tolerable limits for aerial pollutants are unknown. The aim of this experiment was to test the hypothesis that chronic exposure of weaner pigs to controlled concentrations of aerial dust and ammonia lead to slower growth and lower feed intake compared with controls kept in ‘fresh air’.

**Materials and methods** Nine hundred and sixty weaner pigs were exposed for 5½ weeks to controlled concentrations of airborne dust and ammonia in a single, multi-factorial experiment. Performance and health responses were measured but only the former is reported here. The treatments were a dust concentration of either 1.2, 2.7, 5.1 or 9.9 mg m<sup>-3</sup> (inhalable fraction) and an ammonia concentration of either 0.6, 10.0, 18.8 or 37.0 ppm, which are representative of commercial conditions. The experiment was carried out over 2½ years and pigs were used in eight batches, each comprising five lots of 24 pigs. Each treatment combination was replicated once and an additional control group (nominally ≈ 0 mg m<sup>-3</sup> dust and ≈ 0 ppm ammonia) was included in each batch to provide a baseline. For the other four lots in each batch, the dust concentration was common while all four ammonia concentrations were used; thus the split-plot design was more sensitive to the effects of ammonia than dust. The data were analysed by ANOVA with a treatment structure, in GENSTAT notation, of pollutant/(ammonia x dust) where pollutant is a factor of two levels, i.e. control and aerial pollutants.

The pigs were kept separately in five rooms in a purpose-built facility. The pollutants were injected continuously into the air supply. Ammonia was supplied under pressure from a bottle bank and its concentration was measured with a NO<sub>x</sub> chemiluminescent gas analyser after catalytic conversion. The endogenous dust in each room was supplemented by an artificial dust, which was manufactured from feed, barley straw and faeces, mixed by weight in the proportions 0.5:0.1:0.4. The ingredients were oven-dried, milled and mixed and this artificial dust was then resuspended in the supply air. Dust concentration was monitored continuously with a tribo-electric sensor, which was calibrated against an aerodynamic particle sizer and gravimetric samplers.

**Results** Liveweight per pig and cumulative feed intake per pen of 12 pigs were measured after 5½ weeks of exposure. Exposure to both aerial pollutants depressed liveweight relative to the control (control vs pollutant, 25.7 vs 25.0 kg, s.e.d. = 0.33, p = 0.043) and there was a trend for feed intake to be lower for pollutant-exposed pigs (292 vs 280 kg per pen, s.e.d. = 7.1, p = 0.124). The reduction in liveweight was dependent upon the concentration of dust (mean across all ammonia concentrations for increasing dust concentration; liveweight 25.3, 26.4, 24.0 and 24.5 kg, s.e.d. = 0.65, p = 0.081; feed intake 295, 316, 248 and 263 kg per pen, s.e.d. = 14.3, p = 0.016 but not ammonia (mean across all dust concentrations for increasing ammonia concentration; liveweight 24.4, 25.1, 25.3 and 25.3 kg, s.e.d. = 0.41, p = 0.158; feed intake 279, 275, 288 and 279 kg per pen, s.e.d. = 9.0, p = 0.520). There was an interaction between dust and ammonia for liveweight (data not shown, p = 0.030) but the effects were complicated and may have been the result of a Type I error. There was no interaction for feed intake (data not shown, p = 0.210). In general, both feed intake and liveweight gain, but not feed conversion efficiency, were lower for weaner pigs exposed to 5.1 and 9.9 mg m<sup>-3</sup> dust concentration compared with 1.2 and 2.7 mg m<sup>-3</sup> treatments. Other measures of production were also analysed and supported the overall interpretation that dust concentrations of 5.1 mg m<sup>-3</sup> and higher depress performance.

**Conclusion** This study is the first to quantify the effects of aerial pollutant exposure on the performance of weaner pigs. The results suggest that dust concentrations of 5.1 or 9.9 mg m<sup>-3</sup> (inhalable fraction) across ammonia concentrations up to 37 ppm adversely affect performance. The commercial significance of these findings depends on the financial benefits of the superior production at low dust concentrations relative to the costs of providing air of this quality.

**Acknowledgements** This study was financed jointly by the Department for Environment, Food and Rural Affairs, the Meat and Livestock Commission, Acorn House Veterinary Surgery and the National Pig Association.

# Use of visual image analysis for the description of pig growth in size and shape

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**Introduction** Visual imaging systems provide daily plan (overhead) measurements of pigs. These allow monitoring and control of pig growth, as is essential to production efficiency. Schofield *et al.*, 1999 suggest that size measurements can provide accurate estimates of live weight, but a description of growth in terms of size and shape may also give a direct quantification of body form and value. This report presents analyses of the growth of pigs of two commercial breed types in terms of live weight, body plan area and ham width, and examines the relationship between observed body shapes of living pigs and their dissected body composition.

**Materials and methods** Growth trends were analysed using between 70-90 consecutive daily observations for a total of 22 pigs of “Meishan” (25%) and “Pietrain” (50%) commercial types between 68 and 165 days of age. The pigs were fed ad libitum and slaughtered at five approximately equally distant weights through the live weight range of 19 to 139 kg. Daily live weight measurements were obtained from a platform balance integrated into an electronic feeding station (FIRE Feeder, Osborne Europe, Ltd). A visual imaging system placed above the feeding station provided daily the plan area and length measurements of different body parts. Growth curves associated with different measures, pigs and types were compared. Body shape was described by the ratios of ham width (L5, m) to plan (A4, m<sup>2</sup>) area. Relationships were examined between the shape measurements obtained in the living pigs and the related body components obtained by physical carcass dissection.

**Results** For area and ham width measurements over the live weight range considered, growth trends were adequately described by linear functions (Figure 1, and below (with standard errors in brackets)). Auto-regressive models of order one yielded statistically similar slopes and correlation structures for different pigs in each breed, but different intercepts. The differences between the regression slopes associated with different breed types were significant (P<0.05).

Meishan A4 plan area (m<sup>2</sup>) = 0.016 (0.003) + 0.0015 (0.000025) × Age (days)

Meishan L5 ham width (m) = 0.14 (0.002) + 0.0011 (0.000020) × Age (days)

Pietrain A4 plan area (m<sup>2</sup>) = 0.006 (0.002) + 0.0016 (0.000022) × Age (days)

Pietrain L5 ham width (m) = 0.15 (0.002) + 0.0011 (0.000016) × Age (days)

**Figure 1** Growth in the size of Meishan and Pietrain pigs

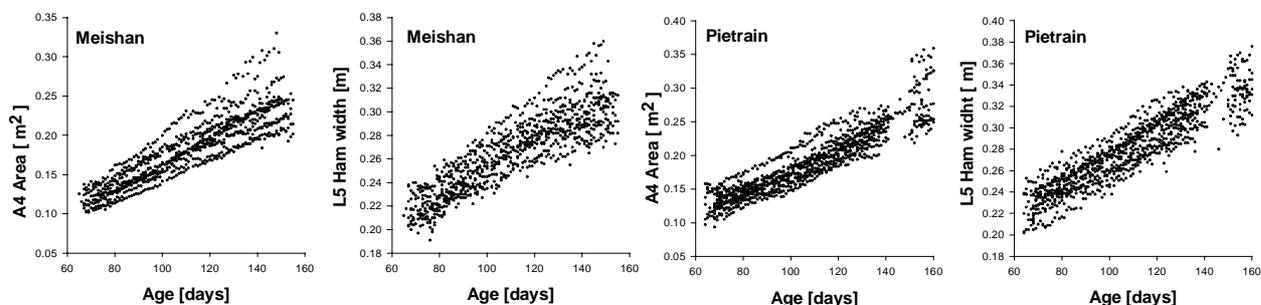


Table 1 shows that there was a strong negative correlation between the L5 ham width relative to A4 surface area and ham fat weight as a proportion of carcass weight. Respective correlations for ham muscle weight were weaker.

**Table 1** Correlations (r) between shape measures in living pigs and weight (wt) of their related dissected parts.

L5 Ham width / A4 Area	Ham wt / Carcass wt	Ham fat wt/ Carcass wt	Ham muscle wt/ Carcass wt
Meishan type	0.60 (P<0.01)	-0.78 (P<0.01)	0.52 (P<0.01)
Pietrain type	0.36 (P<0.01)	-0.71 (P<0.01)	0.25 (P=0.05)

**Conclusions** Visual image analysis (VIA) would appear to promise the means for adequate description of the growth of pigs in size and shape. These dimensions may add significantly to measurement of live weight alone in terms of potential carcass valuation.

**Acknowledgements** This work is part of the UK DEFRA LINK program *Integrated Management Systems for Pig Nutrition Control and Pollution Reduction*. The authors acknowledge the support of DEFRA, MLC, BOCM Pauls Ltd, PIC (UK) Ltd, Osborne (Europe) Ltd.

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# The effect of duration of feeding oilseeds to dairy cows on the persistency of response in milk fatty acid composition

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**Introduction** There is much interest in the manipulation of the fatty acid composition of milk fat so as to improve its health characteristics in the human diet. In an earlier study there was an indication that the concentration of fatty acids in milk may change with time when feeding whole oilseeds rich in polyunsaturated fatty acids (PUFA). In particular a tendency for an increase in CLA and C18:1 and a reduction in C18:2 and C18:3 concentrations with time were seen. The present study was therefore undertaken to examine the changes in the fatty acid composition of milk from high yielding dairy cows fed diets rich in monounsaturated fatty acids (MUFA) or PUFA over an extended period.

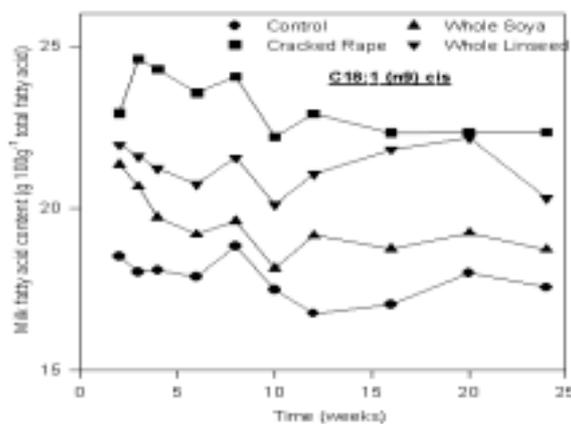
**Materials and methods** Forty Holstein dairy cows were formed into blocks, on the basis of parity (mean 2.5, range 2 to 4) and the number of days in milk (mean 50.3, range 26 to 80) at the start of the experiment, leading to 10 cows per treatment. A one-week covariate period preceded the experimental period. Once allocated to blocks the cows were randomly allocated to one of four treatment diets for a period of 26 weeks. Treatment diets were total mixed rations based on grass silage and containing either either cracked, whole rapeseed (WR; 97 g/kg DM), whole, protected soyabeans (WS; 191 g/kgDM), whole, cracked linseed (WL; 168 g/kg DM) or a control diet (C) containing none of these ingredients. The effects of dietary treatment and feeding duration on milk fatty acid composition were assessed using repeated measures analysis of variance.

**Results** Changes in the fatty acid composition of the milk fat typical of those reported in other studies (e.g. Chilliard *et al.*, 2000) were observed. Regardless of type, the whole oilseeds significantly reduced the saturated fatty acids C14:0 and C16:0 and significantly ( $P<0.001$ ) increased the C18:0 content of the milk fat compared with the control. The cracked rapeseed diet significantly increased C18:1 and conjugated C18:2 (CLA) by 30 and 41% respectively whilst the whole linseed diet significantly increased the C18:3 (n3) content by 233%. The whole soya diet significantly increased the C18:1, C18:2 and C18:3 (n3) compared with the control by 10, 150 and 100% respectively. Both the whole soya and whole linseed significantly increased the CLA content by 59%. For all milk fatty acids, there was a significant effect of time on treatment and many time x treatment interactions. However, many of the changes with time were small and not consistent and the results did not support the earlier observations. Indeed for C18:1, there was a tendency for its concentration to decline with time (Figure 1)

**Table 1** Mean effects on key milk fatty acids (g/100g total fatty acids)

Fatty acid	Diet				s.e.d time
	C	WR	WS	WL	
C16	29.3 <sup>a</sup>	20.6 <sup>b</sup>	23.3 <sup>c</sup>	20.2 <sup>b</sup>	0.315 <sup>***</sup>
C18:1	17.8 <sup>a</sup>	23.2 <sup>b</sup>	19.5 <sup>c</sup>	21.3 <sup>d</sup>	0.368 <sup>***</sup>
C18:3	0.6 <sup>a</sup>	0.7 <sup>b</sup>	1.2 <sup>c</sup>	2.0 <sup>d</sup>	0.028 <sup>***</sup>
CLA	0.39 <sup>a</sup>	0.55 <sup>b</sup>	0.62 <sup>c</sup>	0.62 <sup>c</sup>	0.016 <sup>***</sup>

a,b,c,d, within rows, fatty acid means with different superscripts were significantly different ( $P<0.001$ );  
\*\*\*  $P<0.001$



**Figure 1** Effect of time on C18:1 concentration

**Conclusions** The results do not support the earlier study which showed a tendency for an increase in CLA and C18:1 and a reduction in C18:2 and C18:3 concentrations with feeding duration. In addition, the hypothesis that the rumen gradually adapts to dietary lipid by an increase in biohydrogenation leading to reduced concentrations of PUFA and an increase in partially hydrogenated MUFA is also not supported.

**Acknowledgements** Funding of this work by DEFRA is gratefully acknowledged.

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# Influence of duration of grazing on the fatty acid profile of *M. Longissimus dorsi* from beef heifers

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**Introduction** Current medical advice is to reduce consumption of saturated fat, in favour of polyunsaturated fat, and to increase the consumption of  $\omega$ -3 polyunsaturated fatty acids (PUFAs) at the expense of  $\omega$ -6 PUFAs. Fat of ruminant origin, although it has a nutritionally favourable  $\omega$ -6:  $\omega$ -3 ratio, is rich in saturated fatty acids (SFAs), which have been linked to incidence of coronary heart disease in humans. However, when compared to non-ruminant fat, fat of ruminant origin has a high concentration of conjugated linoleic acid (CLA), which is considered to have anticarcinogenic properties (Belury, 1995). Grass finishing of cattle increased the concentration of CLA and the PUFA:SFA ratio (P:S) when compared with concentrate-based finishing (French et al., 2000). The objective of this study was to investigate the relationship between duration of grass-finishing of cattle and the fatty acid profile of intramuscular lipid.

**Materials and Methods** Sixty Charolais crossbred heifers (BW= 338 kg) were blocked on bodyweight and, within block, randomly assigned to one of four dietary treatments. During a 158-day experimental period, one group of animals remained indoors and were offered a silage/concentrate based diet while another group grazed a predominantly perennial ryegrass pasture. The two remaining groups were initially offered a silage/concentrate diet, but grazed the above pasture for 40 and 99 days prior to slaughter, respectively. Animal growth was monitored throughout the experiment and concentrate and grass allowances were adjusted at three-week intervals to achieve a similar mean carcass weight across all treatments. Animals were slaughtered in a commercial abattoir and carcasses were chilled for 48h at 4°C. Intramuscular fat was extracted in duplicate from samples of *M. longissimus dorsi*, separated into neutral and polar lipids using pre-packed SPE cartridges and then methylated. Fatty acid methyl esters (FAME) were analysed by gas-chromatography with a 100m CP-88 Sil column. The concentration of individual fatty acid in the muscle was determined and the data were analysed according to a randomised block design using Genstat 6.0.

**Results** The fatty acid concentrations of the neutral lipid and the polar lipid fractions are summarized in Table 1. In both lipid fractions, extending the grazing period resulted in a linear increase in the concentration of linolenic acid, CLA (c9t11 isomer) and trans-vaccenic acid (TVA) and a linear decrease in the  $\omega$ -6: $\omega$ -3 ratio. The P:S ratio and CLA t10c12 concentration was increased linearly in the neutral lipid fraction, but was not altered in the polar lipid fraction with extended grazing prior to slaughter.

**Table 1** Concentration of fatty acids in *M. longissimus dorsi*

	Neutral lipids (g/100g FAME <sup>+</sup> )						Polar lipids (g/100g FAME)					
	Days at grass						Days at grass					
	0	40	99	158	s.e.d.	P	0	40	99	158	s.e.d.	P
C 18:2	1.23	1.31	1.20	1.21	0.07	ns	13.56	15.35	11.99	12.05	0.99	**L
C 18:3	0.55	0.64	0.71	0.84	0.04	***L	2.86	5.06	5.34	5.92	0.35	***L,Q
CLAc9t11	0.53	0.53	0.60	0.78	0.06	***L	0.19	0.23	0.27	0.31	0.03	**L
CLAt10c12	0.03	0.04	0.07	0.07	0.01	***L	0.02	0.02	0.02	0.02	0.005	ns
TVA	1.44	2.09	2.47	3.37	0.20	***L	0.26	0.58	0.58	0.62	0.13	*L
P:S Ratio	0.06	0.07	0.07	0.08	0.004	***L	1.00	1.15	0.97	1.08	0.09	ns
$\omega$ -6: $\omega$ -3 Ratio	1.97	1.74	1.71	1.38	0.16	**L	2.41	2.13	1.68	1.50	0.11	***L

<sup>+</sup> FAME: Fatty Acid Methyl Esters; \* = <0.05, \*\* = <0.01, \*\*\* = <0.001; L=Linear, Q= Quadratic

**Conclusions** The results of this study indicate that the beneficial effect on the fatty acid profile of muscle from cattle offered grazed grass is strongly dependent on the duration of the grazing period. Further research is needed on strategies to optimize the concentration of beneficial fatty acids in grass-based production systems.

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**Acknowledgements** This study was supported by the European Commission (QLRT-2000-31423)

# An evaluation of the effect of concentrate proportion of the diet during previous and present lactations on animal performance of two breeds of lactating dairy cows

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**Introduction** Studies undertaken to evaluate long responses to concentrate feeding, normally assess the direct effects (i.e. the effects during the period of increased feeding). However in addition to the direct responses to concentrate feeding during the experimental period, it has been recognised that additional nutrients fed during one stage of lactation may result in improvements in animal performance in later lactation or in the subsequent lactation. Keady *et al.* (2002) reported that the milk yield response to concentrate proportion in the diet differed between Holstein (HF) and Norwegian (NC) dairy cattle with responses of 1.4 and 0.8 kg corrected milk/kg concentrate dry matter (DM) respectively. The objective of the present study was to evaluate the effects of concentrate proportion in the diet in the previous lactation on animal performance in the subsequent lactation. The effect of concentrate proportion in the diet on animal performance of HF and NC dairy cattle was also evaluated.

**Materials and Methods** Twenty-four animals from the HF and NC breeds which had completed either one or two lactations, and had been offered either a high (H) or low (L) concentrate proportion diet in their previous lactation were assigned to the present study. Within each breed, half of the animals on each concentrate proportion diet changed diet immediately post-calving whilst the others remained on the same diet giving a total of eight treatments. Within each breed the treatments were as follows: HH, HL, LH and LL, the first letter of the treatment denotes the concentrate proportion of the diet in the previous lactation whilst the second letter denotes the concentrate proportion of the diet in the present lactation. During the previous and present lactations the high concentrate proportion diets had concentrate proportions of 0.65 and 0.60; 0.55 and 0.50; and 0.45 and 0.40 whilst the low concentrate proportion diets had concentrate proportions of 0.35 and 0.30; 0.25 and 0.20; and 0.15 and 0.10 for the first, second and third trimester of lactation respectively. All cows received 180 g/d of a mineral/vitamin pre-mix. 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. 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. The study was analysed as a 2x2x2 factorial design experiment using Genstat ANOVA.

**Results** The silage offered in the present study had a pH, predicted D-value and concentrations of DM, crude protein and ammonia nitrogen (N) of 4.0, 710 g/kg DM, 266 g/kg, 163 g/kg DM and 108 g/kg N respectively. The effects of breed and concentrate proportion of the diet during the previous and current lactations on animal performance are presented in Table 1. The HF breed had significantly higher food intake ( $P<0.001$ ), yields of milk ( $P<0.001$ ) and fat plus protein ( $P<0.001$ ), milk energy output ( $P<0.01$ ) and concentration of fat ( $P<0.05$ ). Increasing the concentrate proportion in the present lactation increased food intake ( $P<0.001$ ), the yields of milk ( $P<0.001$ ) and fat plus protein ( $P<0.001$ ) and milk protein concentration ( $P<0.001$ ). The treatment HL had significantly higher fat concentration relative to treatment LH. There were significant breed by treatment interactions for the yields of milk and fat plus protein. For the NC breed the HH and HL treatments tended to have lower yields of milk and fat plus protein relative to LH and LL treatments respectively. However for the HF breed the yields of milk and fat plus protein were similar for the HH and HL treatments whereas the HL treatment tended to increase output relative to the LL treatment. There tended ( $P=0.08$ ) to be a breed by treatment interaction for milk fat concentration. For the HF breed increasing the plane of nutrition in the previous lactation tended to increase fat concentration whereas for the NC breed increasing the plane of nutrition in the present lactation tended to decrease it.

**Table 1** The effect of breed and concentrate proportion treatment on animal performance (weeks 1-16)

Treatment (T)	Breed (B)								Sem	B	T	BxT
	Holstein				Norwegian							
	HH	HL	LH	LL	HH	HL	LH	LL				
Tot. DMI (kg/d)	19.0 <sup>cd</sup>	16.4 <sup>b</sup>	19.8 <sup>d</sup>	15.7 <sup>b</sup>	18.2 <sup>c</sup>	14.0 <sup>a</sup>	18.0 <sup>c</sup>	15.6 <sup>b</sup>	0.46	***	***	0.07
Milk yield (kg/d)	38.5 <sup>c</sup>	33.6 <sup>b</sup>	38.4 <sup>c</sup>	31.1 <sup>b</sup>	32.0 <sup>b</sup>	27.8 <sup>a</sup>	33.7 <sup>b</sup>	30.5 <sup>a</sup>	1.08	***	***	*
Fat (g/kg)	42.9 <sup>bc</sup>	42.7 <sup>bc</sup>	41.6 <sup>bc</sup>	41.5 <sup>bc</sup>	40.4 <sup>ab</sup>	43.4 <sup>c</sup>	38.3 <sup>a</sup>	41.7 <sup>bc</sup>	0.88	*	*	0.08
Protein (g/kg)	34.2 <sup>d</sup>	31.3 <sup>ab</sup>	34.4 <sup>d</sup>	32.3 <sup>abc</sup>	33.1 <sup>bcd</sup>	30.6 <sup>a</sup>	33.5 <sup>cd</sup>	32.3 <sup>abc</sup>	0.64	NS	***	NS
Fat + Prot. (kg/d)	2.91 <sup>d</sup>	2.50 <sup>c</sup>	2.89 <sup>d</sup>	2.29 <sup>bc</sup>	2.36 <sup>bc</sup>	2.04 <sup>a</sup>	2.44 <sup>bc</sup>	2.23 <sup>ab</sup>	0.076	***	***	**
Milk energy (MJ/kg DMI)	6.5 <sup>bc</sup>	6.7 <sup>c</sup>	6.3 <sup>b</sup>	6.4 <sup>bc</sup>	5.5 <sup>a</sup>	6.5 <sup>bc</sup>	5.9 <sup>ab</sup>	6.1 <sup>ab</sup>	0.24	**	NS	NS
CS at calving	2.99 <sup>a</sup>	2.94 <sup>a</sup>	2.59 <sup>a</sup>	2.65 <sup>a</sup>	4.09 <sup>c</sup>	3.96 <sup>c</sup>	3.45 <sup>b</sup>	3.51 <sup>b</sup>	0.155	***	**	NS

**Conclusions** The HF cows produced greater quantities of milk and utilised nutrients more efficiently for milk production relative to the NC cows. Concentrate proportion in the present lactation had the greater effect on animal performance. However when the high concentrate proportion diet in the previous lactation and low concentrate proportion in the present lactation were offered, milk yield was increased by 286 kg and decreased by 305 kg for the HF and NC cows respectively during the first 16 weeks of lactation, reflecting differences in the condition score of the animals at calving.

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## A comparison of once- versus twice-daily milking on performance of late lactation dairy cows

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**Introduction** Labour constitutes a major and increasing cost on dairy farms, while at the same time, the availability of skilled labour is decreasing. In addition, younger farmers are placing an increasing emphasis on lifestyle. Consequently, there is an increased interest in milk production systems involving reduced labour inputs. In view of the fact that approximately 33% (O'Brien *et al.*, 2002) of the daily labour input on dairy farms is associated with the milking routine (herding, milking and washing), the adoption of once daily milking would appear to offer considerable potential to reduce labour inputs. This study examined the effect of once-daily milking, in late lactation, on animal performance.

**Material and methods** The study involved fifty multiparous (mean lactation number, 2.6 (s.d., 0.61)), autumn calving, Holstein/Friesian dairy cows. Forty-four of the animals were pregnant. Animals were divided into two treatment groups (ODM and TDM) five days prior to the start of the study, with animals being 232 (s.d. 24.2) days calved when the study commenced on 10<sup>th</sup> June. From 10<sup>th</sup> June onwards, animals on treatment ODM were milked once daily, in the morning, while with treatment TDM, animals were milked twice daily (morning and evening), as per pre-experimental practice. Animals were managed on these two different milking regimes until drying off, with drying off planned 8 weeks pre-calving. However, a number of animals were dried off early when mean weekly milk yields fell below 5.0 kg/day. Although the two experimental groups were balanced in relation to the mean planned drying-off date (86 days post start of the study), as a consequence of animals being dried off early, the actual mean number of days on the study for the ODM and TDM groups were 77 and 81 respectively. The six non-pregnant animals remained on the study for 79 days, the mean number of days for all animals on the study. Throughout the study the two experimental groups were grazed on adjacent plots, with the area of fresh herbage offered daily being the same for both groups. During the study all animals were offered 3.0 kg of concentrate daily, with the ODM group receiving this in a single feed during morning milking, while the concentrates offered to the TDM group were split between two equal feeds each day (1.5 kg at each milking).

**Results** Mean pre- and post grazing sward heights were 10.8 (s.d. 1.97) and 5.9 (s.d. 1.27) cm for the ODM group and 10.8 (s.d. 2.06) and 6.0 (s.d. 1.27) cm for the TDM group. Animal performance data were analysed by ANOVA, with data from the pre-experimental week being used as a co-variate for all parameters, with the exception of somatic cell count. Animals on the ODM regime had a 23% lower total milk output and daily milk yield ( $P \leq 0.01$ ) compared to those on the TDM regime, while milk fat, protein and energy concentrations were greater with the ODM ( $P < 0.001$ ). However milk fat + protein yield was only 19% lower with the ODM milking regime compared to the TDM milking regime. Milking regime had no significant effect on either live-weight or condition score at drying off ( $P > 0.05$ ).

*Table 1 Effect of once vs twice daily milking on animal performance*

	ODM	TDM	SEM	SIG
Total milk output (kg)	939	1224	60.9	**
Milk yield (kg/day)	11.9	15.4	0.39	***
Milk composition				
Fat (g/kg)	47.1	43.3	0.61	***
Protein (g/kg)	38.6	36.2	0.37	***
Lactose (g/kg)	45.2	46.8	0.22	***
Energy (MJ/kg)	3.46	3.30	0.029	***
Fat + protein yield (g/day)	1003	1233	33.3	***
Milk energy output (MJ/day)	40.7	51.1	1.38	***
Live-weight at drying off	630	626	6.4	NS
Condition score at drying off	2.5	2.4	0.03	NS

**Conclusions** Although the adoption of once-daily milking in late lactation resulted in a reduction in milk yield of approximately 23%, milk fat and milk protein concentrations were increased with once-daily milking. However, animals managed on a once-daily milking regime did not gain live-weight, or increase in condition score, compared to those managed on a twice-daily milking regime. Nevertheless, once-daily milking offers the potential to make considerable savings in labour costs.

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## Effects of habituation to the milking parlour on production, health and fertility of Norwegian and Holstein dairy herd replacements

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**Introduction** During the transition period (defined as three weeks prior to calving to three weeks post-calving) heifers are exposed to physiological, nutritional, management, and social changes as they enter the dairy herd. One obvious change is the introduction of heifers to the milking parlour. Previous work has shown that when mature cows were milked in unfamiliar surroundings, milk yield, milk flow rate and milking duration were affected due to increased stress (Rushens, *et al.* 2001). The objective of the current study was to investigate the effects of habituating heifers to the milking parlour pre-calving on the subsequent performance of Holstein-Friesian and Norwegian dairy herd replacements.

**Materials and Methods** Fifty-four spring calving heifers (32 Holstein-Friesian and 22 Norwegian (NRF) dairy cattle) were used in a study to investigate the effects of habituating heifers to the milking parlour prior to calving. The average genetic merit for Holstein-Friesian and Norwegian replacements were 35.9 (s.d. 5.79) PTA<sub>2000</sub> and 11.7 (s.d. 2.17) Total Merit Index respectively. Animals were grouped according to genotype, predicted calving date, live weight and genetic merit into either of two treatments. Treatment 1, animals were introduced to the milking parlour (20-point rotary herringbone parlour) 3-weeks prior to calving (Habituation group), whereas Treatment 2 animals were introduced to the parlour post-calving (Control group). Prior to calving heifers were housed in two groups in adjacent cubicles within the same building. Habituation heifers received 1 kg/head/d of concentrate while in the parlour, the control heifers were offered 1 kg/head/d concentrate along the feed passage in the cubicle accommodation, with all heifers offered grass silage *ad libitum*. Post-calving heifers were offered 6 kg/head/d concentrate along with grass silage *ad libitum* within a total mixed ration, and 4 kg/head/d concentrate in the parlour. The data were analysed using repeated measures REML analysis and fixed effects included week of lactation, breed, treatment and their interactions.

**Results** Holstein-Friesian heifers yielded more milk, fat, protein and lactose per day during early lactation than the Norwegian heifers ( $P < 0.001$ ). Milk fat concentration was higher for Holstein-Friesian heifers ( $P < 0.001$ ), but there was no significant difference between milk protein and lactose concentrations for the two breeds (Table 1). Average and peak milk flow rates were higher for the Holstein-Friesian compared with Norwegian heifers ( $P < 0.001$ ). Somatic cell counts were greater for Holstein-Friesian heifers. Heifers in the habituation treatment yielded 1.5 kg/d more milk compared with heifers in the control group ( $P < 0.001$ ). The duration of milking was longer and milk flow rates were significantly slower for heifers in the habituation group compared with the control group ( $P < 0.001$ ). Somatic cell counts were lower for the habituation treatment. The interval from calving to first observed oestrus was longer for the Holstein-Friesian compared with the Norwegian heifers ( $P < 0.01$ ), and the interval to conception was longer ( $P < 0.05$ ) for heifers on the habituation treatment.

**Table 1:** Production and milking traits of first calving Holstein-Friesian and Norwegian heifers during the first 100 days of lactation by breed and treatment

	Breed				Treatment			
	Holstein	Norwegian	s.e.d	sig	Control	Habituation	s.e.d	sig
<b>Milk Production</b>								
Milk Yield (kg/d)	27.4	25.0	0.27	***	25.4	26.9	0.27	***
Fat Concentration (g/kg)	40.7	37.9	0.60	***	39.9	38.7	0.60	*
Protein Concentration (g/kg)	33.2	32.9	0.28	NS	33.0	33.1	0.28	NS
Lactose Concentration (g/kg)	50.2	50.3	0.15	NS	50.3	50.2	0.15	NS
Somatic Cell Count (log <sub>10</sub> )	1.779	1.669	0.0366	**	1.785	1.663	0.0366	***
<b>Milk Flow Characteristics</b>								
Duration of Milk Flow (min)	5.96	6.02	0.109	NS	5.68	6.31	0.109	***
Milk Flow Rate (kg/min)	2.37	2.17	0.041	***	2.35	2.20	0.041	***
Peak Milk Flow Rate (kg/min)	3.52	3.24	0.078	***	3.53	3.24	0.078	***
<b>Reproductive Performance</b>								
Days to 1 <sup>st</sup> Progesterone Rise (d)	38.3	29.7	4.46	NS	36.2	31.8	4.46	NS
Days to 1 <sup>st</sup> Oestrus Detection (d)	59.9	43.1	6.22	**	49.2	53.8	6.22	NS
Days to Conception (d)	96.1	89.1	9.22	NS	83.0	102.2	9.22	*
Services/Conception	1.61	1.57	0.303	NS	1.29	1.89	0.303	NS

**Conclusion** Allowing heifers to become accustomed to the milking parlour prior to first calving increased milk production and reduced somatic cell counts while increasing the interval to conception.

**Reference** Rushen, J., Munksgaard, L., Marnet, P.G. and DePassille, A.M., 2001. Human contact and the effect of acute stress on cows at milking. *Applied Animal Behaviour Science* **73**: 1-14.

## Prediction of dairy cattle reproductive performance in pastoral feeding systems

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**Introduction** As NZ milk production is predominantly seasonal and reliant on pasture growth, a high pregnancy rate within a short time after planned start of mating is essential to match feed supply to production. A Holstein-Friesian strain trial was established in Hamilton, New Zealand, to investigate the physical and financial performance of three strains of dairy cattle under a range of pasture based systems that differ in stocking rate and supplementary feeding. The three strains of cows were: Overseas High genetic merit (OS), New Zealand High genetic merit (NZH) and New Zealand Low genetic merit (NZL). The objective of this study was to predict reproductive performance from information available prior to mating, as an aid to on-farm decisions.

**Materials and methods** Data were on 67 NZH and 67 OS heifers and 36 NZL heifers calving from mid July 2001 to late September 2001. Eleven farmlets were established by allocating heifers from OS and NZH strains to four groups per strain and heifers from NZL into three groups. The eleven groups were kept under a range of different feeding systems. Feed allowance (pasture plus supplements) ranged from 4.5 to 7.0 tDM/cow/yr. Milk yield (MY) and fat and protein percentages (F%, P%) were measured weekly and live weight (LW) and body condition scores (CS) taken weekly until mating and then fortnightly, monthly means were calculated from each of these. CS was measured on a scale of 1 to 10, with 1 being extremely thin, 10 being obese and 5 optimum for calving cows. Reproductive measures were cows in calf at 42, 49 and 56 days after the planned start of mating (October 2<sup>nd</sup>); P42, P49 and P56 respectively. All NZH and NZL cows conceived and 11 OS heifers failed to become pregnant at all. Data were analysed using JMP version 5 (SAS, 2002). Three methods of predicting P42, P49 and P56 were used: nominal logistic regression (LR), discriminant analysis (DA) and partition analysis (PA). Partition analysis is often used as a data mining technique, because it is good for exploring relationships without having a good prior model. It is often used as a diagnostic tool for analysing symptoms and diagnoses of a given illness to provide a hierarchy of questions in order to provide a quick initial diagnosis. Partition analysis recursively sub-divides data creating a tree of partitions. Each split is chosen to maximise the difference in the responses between the two branches of the split, the most significant split is determined by the largest likelihood-ratio chi-square statistic. A maximum of 12 splits were done per partition analysis. The predictors used in our analyses were: average monthly CS, LW, MY, F%, P% for the first three months of lactation, CIDR progesterone insert (0/1), feed allowance, % North American genetics (%Hol) and calving week (from first calving). Predictions were compared to phenotypes using contingency analysis for all three models.

**Results** CS change from month 1 to 2 or 2 to 3 was less important as a predictor of pregnancy than average CS score. Also fat and protein yields and the ratio of fat to protein were not as informative as F% and P%. The mean %Hol was 23%, 7.5%, 91% for NZH, NZL and OS. It is interesting that in our analysis %Hol did not emerge as an important predictor, yet NZL was 16% higher than OS for P42. However, other factors, such as CS, weeks in milk, P% and F% were important, these characterise differences between the strains and pregnancy status. CS in month 3 and calving week were consistently important predictors of the P42, P49 and P56 in all analyses. Partition analysis was the best method of prediction (Table 1). Kappa values, measuring the degree of agreement between observations and predictions ranged between 0.29 and 0.38 for discriminant analysis, 0.36 and 0.42 for logistic regression and 0.81 and 0.85 for partition analysis.

**Table 1** Kappa values and percentage of incorrectly classified animals for each of the models tested

Method	Logistic Regression			Discriminant Analysis			Partition Analysis		
	P42	P49	P56	P42	P49	P56	P42	P49	P56
Trait									
Kappa	0.42	0.36	0.36	0.29	0.32	0.38	0.81	0.84	0.85
% Incorrectly classified	26	24	20	33	32	28	9	7	7

The partition model predicted that all cows with CS  $\geq 4.75$  in month 3 were pregnant by day 49 and 56 (data set mean of CS in month 3 was 4.25 and SD was 0.49). One cow out of 45 did not fit this rule for P42. All cows with CS  $< 4.75$  in month 3 and calving after week 4 from start of calving failed to get in calf by day 42. The first four splitting rules were the same for P42, P49 and P56.

**Conclusions** Partition analysis provides a useful way of identifying key factors affecting any trait of importance. The method can be used to provide management guidelines. Cows that were in-calf early had higher than average condition scores and calved before week 4 after start of calving.

**Acknowledgements** This work was funded by NZDB Global Programme funding and Livestock Improvement Corporation.

## Postpartum anovulatory intervals in two genotypes of pasture-fed Holstein-Friesian dairy cattle

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**Introduction** In pasture-based dairying systems it is important to maintain a 365-day calving interval, which requires that cows have a rapid resumption of postpartum ovulatory activity and high conception rates. The major form of infertility in New Zealand (NZ) dairy cows is an extended postpartum anovulatory interval (ppai; Macmillan, 2002), a problem that can be exacerbated by low body condition score (BCS) at calving (McDougall, 1995). Furthermore, Holstein cows, originating from North American genetic strains (Overseas; OS), which have been widely used in NZ in recent years, have longer intervals to first mating and conceive later than do NZ strains, suggesting a possible delay in the initiation of postpartum cyclicity (Harris and Winkleman, 2000). Worldwide, there is concern over an apparent decline in the fertility of Holstein-Friesian dairy cattle (Butler *et al.*, 1995; Royal *et al.*, 2000). The purpose of this trial was to determine whether there was a difference in ppai and BCS between NZ and OS Holstein Friesians that may affect reproductive performance.

**Materials and methods** One hundred and twenty five NZ animals of high genetic merit (average of 23% OS genetics), and 123 OS high genetic merit animals (average of 89% OS genetics) that calved within 6-weeks of the start of the calving season in Years 1 (all two-year-olds) and 2 (two- and three-year-olds) were included in the study. Animals were run in 8 herds, with feeding allowances ranging from 5 to 7-tDM per cow per year of pasture or pasture plus maize silage. Twice weekly whole milk samples were collected and progesterone concentrations determined using an enzyme-linked immunosorbant assay kit (Ridgeway Sciences, Gloucestershire, UK). Luteal activity was defined as progesterone concentrations of >2ng/ml followed by >3ng/ml in two consecutive samples, with an adjustment for errors associated with twice weekly sampling of -1.8 days. Body condition scores were assessed on a 10-point scale (1= emaciated, 10 = obese; Macdonald, 1993). Postpartum anovulatory intervals were analysed using parametric frailty models with normal distribution in which feeding level, age at calving (and year where applicable) and strain were included as fixed effects and sire as a random effect using the *suvReg* function in *S Plus 6.1*. Proportional data were analysed using generalised linear models with binomial error distribution.

**Results** Data is presented in Table 1. In Years 1 and 2 ppai were significantly ( $P<0.005$ ) shorter in OS than in NZ animals. There was no difference in BCS at calving or BCS loss between strains. Significantly ( $P=0.001$ ) more NZ animals were treated for anoestrus prior to the start of mating in Year 1, but this difference was no longer significant in Year 2 ( $P=0.055$ ). However, despite the relatively earlier resumption of postpartum ovulatory activity significantly ( $P<0.05$ ) more NZ animals conceived during a 12-week mating period (92% vs. 78%) in Year 1 of the trial.

**Table 1** Body condition score and reproductive outcomes in OS animals, presented as difference compared to NZ animals.

	BCS at calving	BCS change from calving to 4 weeks	Postpartum anovulatory interval	Treated for anoestrus	12-week pregnancy rate
Year 1	-0.23 units	0.08 units	-20 days	-36%	-14%
Year 2	-0.20 units	0.06 units	-15 days	-11%	Not yet available

**Conclusions** The results of this study suggest that OS heifers initiate ovarian cycles earlier in the postpartum period than NZ heifers, despite similar BCS at calving, and similar BCS loss after calving. This resulted in more NZ animals requiring treatment for anoestrus. The fertility of these animals now needs to be compared to determine whether earlier resumption of ovarian activity, and fewer treatments for anoestrus translates into more pregnancies. Initial results indicate that despite earlier resumption of postpartum ovulatory activity, and fewer animals treated for anoestrus that the pregnancy rate of OS animals is lower than NZ animals.

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# The effect of nutrition on nematode faecal egg output in lactating, organically managed ewes.

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**Introduction** Nematodes are a particular challenge to animal health and productivity in organic sheep systems, where the prophylactic use of anthelmintic is prohibited. The peri-parturient rise in faecal egg output, a consequence of relaxation of host immunity in late pregnancy and early lactation, is potentially a key factor in the epidemiology of parasitic gastro-enteritis on organic sheep farms. Coop and Kyriazakis (1999) developed a hypothesis to explain the relationship between nutrition and periparturient breakdown of immunity to parasites, and there is now an increasing body of evidence for the involvement of dietary protein (Houdijk et al 2001). The objective of this study was to test this hypothesis in organically managed ewes carrying a mixed, naturally acquired infection, grazing on grass/clover in early lactation, and to examine the potential for a nutritional approach to parasite control in commercial practice.

**Materials and Methods** Forty eight twin-bearing Scottish Blackface ewes were managed to achieve a body condition score of 2-2½, one week before lambing (mean live weight 60.4 kg). After lambing, ewes were blocked for lambing date, body condition score, faecal egg count and liveweight and allocated to one of two treatments 1) grazing alone, or 2) grazing plus 0.6 kg per day freshweight per ewe of expelled non-GM soyabean meal. Stocking rate at pasture was 18 ewes per hectare, and sward height was maintained within a target range of 4 – 6 cm for all but the last 10 days of the experiment. Ewe live weight, ewe body condition score, and lamb live weight were taken weekly. Faecal egg counts (FEC, in eggs per gram (epg) fresh faeces) were assessed weekly. Ewes were ultrasonically scanned for backfat and muscle depth at fortnightly intervals from two weeks after lambing. Feed intake was estimated during week 5 of lactation using N-alkane boluses (Captec ®). Performance data were analysed by repeated measures ANOVA in GENSTAT. FEC data were log-transformed, and are reported as backtransformed means with 95% confidence intervals.

**Results** Supplementation resulted in an increased intake of energy (24 vs. 32 MJ/day) and metabolisable protein (222 vs. 399 g/day), and improved ewe live weight (Figure 1;  $P < 0.001$ ) and muscle depth ( $P < 0.01$ ). Unsupplemented ewes also tended to have lower body condition scores ( $P = 0.091$ ). There were no statistically significant differences in backfat depth. Supplemented ewes tended to have higher litter weights at 8 weeks of age ( $P = 0.10$ ). Until parturition, mean faecal egg count (FEC) varied between 200 and 300 eggs per gram of fresh faeces. Based on repeated measures analysis, there was no overall effect of supplementation during the entire experiment ( $P = 0.25$ ). However, FEC of the control ewes showed a clear temporal increase in FEC during weeks 4-6 of lactation. This rise was not observed in supplemented ewes, resulting in statistically significant differences in FEC over this period (Figure 2;  $P < 0.01$ ).

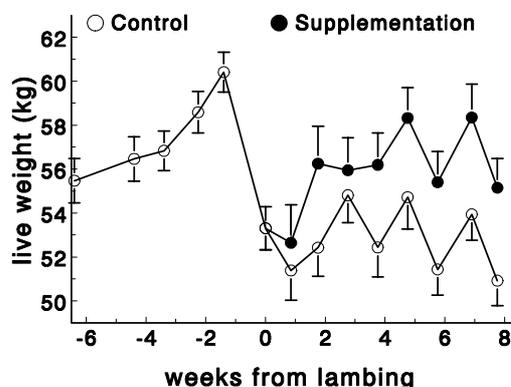


Figure 1: Ewe live weight (kg)

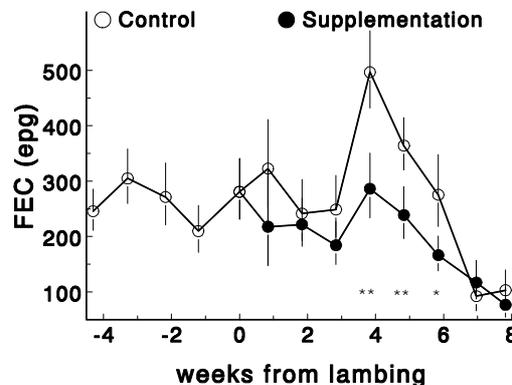


Figure 2: Ewe FEC (epg)

**Conclusions** This experiment attempted to balance conditions likely to test the experimental hypothesis, with conditions relevant to UK organic sheep production systems. The results support the view that under those conditions, nutritional manipulation can improve immunity to gastrointestinal parasites in lactating ewes. However, the observed effects on FEC were less marked than in earlier studies with Halfbred ewes (Houdijk et al., 2001), and appeared only during the period of assumed peak lactation. The data suggests that the level of nutrition provided to the control sheep, although challenging, may not have been sufficiently low to result in a substantial breakdown in host immunity.

**Acknowledgements** DEFRA funding for this work is gratefully acknowledged.

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## Reducing the degree of protein scarcity rapidly increases immunity to nematodes in ewes

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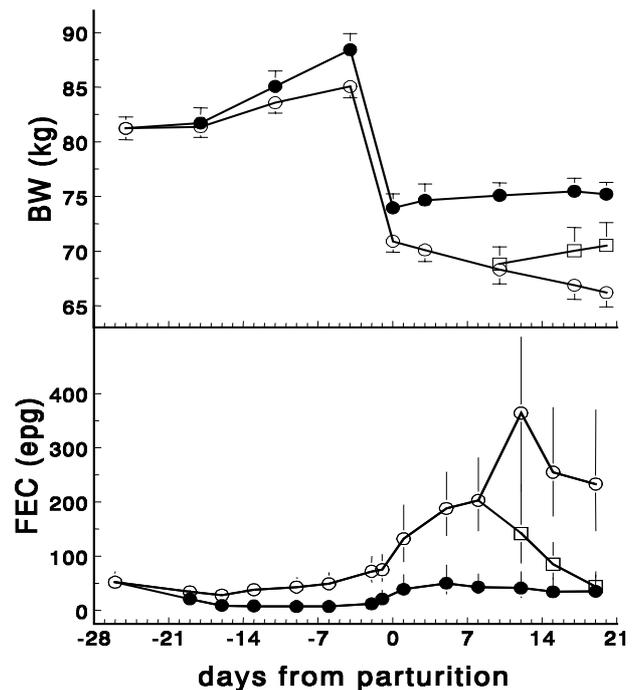
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**Introduction** There is an increasing body of evidence to support the view that the periparturient breakdown of immunity to parasites has a nutritional basis; an increased supply of metabolizable protein (MP), at times of MP scarcity, would reduce gastrointestinal nematode egg excretion and worm burdens in periparturient ewes (Houdijk et al., 2001). However, the rate at which MP supply can improve expression of immunity to gastrointestinal nematodes is not known. The objective of this experiment is to assess this rate, which will be studied through reducing the degree of MP scarcity.

**Materials and Methods** Three groups of 15 twin-bearing Greyface ewes were trickle infected with 10,000 infective *Teladorsagia circumcincta* larvae per day, three days per week, from day<sub>-70</sub> onwards (relative to expected parturition). One group of twin-rearing ewes was fed daily allowances that were calculated to supply 0.85 and 0.6 times the MP requirements during late pregnancy and lactation, respectively (L22). A second group of twin-rearing ewes was fed daily allowances calculated to supply 1.25 and 1.2 times these MP requirements, respectively (H22). The third group was fed the same daily allowances as the L22 ewes until the end of the experiment, but one lamb was weaned at d<sub>10</sub> (L21). Thus, feeding treatments for L22 and L21 ewes were similar until d<sub>10</sub>. All daily allowances were calculated to provide 0.90 times the metabolizable energy (ME) requirement for twin-rearing ewes (AFRC, 1993). The assumptions for estimating MP and ME requirements were a litter birth weight of 10.3 kg, no maternal bodyweight gain during pregnancy, a mean milk production of 3.7 kg per day, a bodyweight loss of 100 g/day during lactation and 10.2 g MP per day for wool growth. Ewes and lambs were weighed once or twice weekly, respectively, and ewe faecal egg counts (FEC, in eggs per gram fresh faeces, epg) were assessed twice weekly. FEC were log-transformed, and reported as backtransformed means with 95% confidence intervals. ANOVA was used to assess the treatment effects on ewe and litter body weight gain, litter birth weight and FEC. The ANOVA for FEC was included in repeated measures analysis.

**Results** Figure 1 shows the mean body weight (BW) and FEC of the ewes around parturition. Compared to the H22 ewes, L ewes grew slower during late pregnancy ( $P < 0.05$ ) and were lighter immediately post parturition ( $P < 0.01$ ). During lactation, the L ewes lost BW until d<sub>10</sub> whilst the H22 ewes maintained BW ( $P < 0.001$ ). From d<sub>10</sub> onwards, the trends in BW changes continued for the L22 and H22 ewes, whilst the L21 ewes started to gain BW. On average, the litters of the L ewes were lighter at birth than those of the H22 ewes (9.9 vs 10.6 kg, s.e.d. 0.39;  $P = 0.087$ ), and grew slower until d<sub>10</sub> (666 vs 804 g/d; s.e.d. 33.2;  $P < 0.001$ ). From d<sub>10</sub> onwards, the L22, L21 and H22 litters gained 623, 498 and 731 g/d (s.e.d. 29.2;  $P < 0.001$ ). Time and feeding treatment significantly interacted for FEC ( $P < 0.05$ ). From d<sub>-9</sub> to d<sub>10</sub>, FEC of the L ewes were higher than that of H22 ewes. From d<sub>10</sub> onwards, FEC of L22 ewes remained higher than that of H22 ewes. However, FEC of the L21 ewes decreased after d<sub>10</sub>, and were similar to H22 ewes from d<sub>15</sub> onwards.

**Conclusion** The results of this study indicate that reducing the degree of protein scarcity rapidly improves expression of immunity to gastrointestinal nematodes in lactating sheep. The data support the view that nematode egg excretion is relatively sensitive to the degree of MP scarcity. This points towards the possibility of rapidly reducing the periparturient ewes' contribution to pasture contamination through reducing MP scarcity by improved protein nutrition.



**Figure 1.** Body weight and FEC of the lactating ewes.

○: Low MP supply, twin-rearing (L22).

●: High MP supply, twin-rearing (H22).

□: As L22 but one lamb weaned at d<sub>10</sub> (L21).

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

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# The effects of metabolisable protein on the periparturient relaxation of immunity against *Teladorsagia circumcincta* in mature Friesland dairy ewes

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**Introduction** With increasing incidence in anthelmintic drench resistance, it is appropriate that more sustainable approaches to controlling nematode infections in livestock should be investigated. It is believed that larval contamination of pasture originates from the mature breeding ewe during the periparturient breakdown of immunity. Donaldson *et al.* (1998) reported that increasing the metabolisable protein (MP) supply of the ewe in late pregnancy may moderate the periparturient relaxation of immunity. The most pronounced results to date are those involving the supplementation of diets with fishmeal, believed to be related to the 'protected' protein content of the ration. Fishmeal increases both MP and polyunsaturated fatty acid content of the diet but as yet it is unclear which of these components of the diet may affect immunity to parasites. This study investigated the role of MP supply in maintaining immunity to parasitic infection in pedigree Friesland dairy ewes.

**Materials and methods** Thirty-two pregnant Friesland ewes were fed 1 of 2 dietary treatments and allocated to 1 of 4 groups: Basal MP, non infected (BMP N), Basal MP, infected (BMP I), High MP, non infected (HMP N) or High MP, infected (HMP I) in a 2x2 factorial design (n=8), from 5 weeks prior to parturition through to week 11 of lactation. The diets offered, differed in levels of MP, a Basal MP diet (0.99 x daily requirement) and a High MP diet (1.74 x daily requirement). From week 3 *pre-partum* the infected treatment ewes received approximately 2000 *Teladorsagia circumcincta* infective larvae per day. Lambs were weaned 72 hours *post-partum* and ewes machine milked 3 times per day until week 7 then twice daily until week 11. Faecal samples were collected weekly for faecal egg count (FEC) determination, together with blood samples which were obtained for analysis of immunological and nutritional parameters. Milk yields were recorded weekly and samples collected for determination of fat and protein. Liveweight and condition scores (CS) were recorded weekly. The ewes were slaughtered between weeks 6-7 and 10-11 *post-partum* to determine abomasal worm burdens. The FECs and worm burdens were transformed according to Log10 (FEC+1) prior to statistical analysis using GENSTAT.

**Results** Supplemented levels of MP had no significant effect on worm burdens or FECs. There were no significant differences between the mean ewe weights, CS or lamb birthweights. *T. circumcincta* challenge significantly increased the eosinophil counts (P=0.002). The HMP treated ewes produced significantly higher mean daily milk yields than the BMP treated ewes (P=0.011) and by increasing the MP supply the milk fat content was lowered significantly (P=0.02) but there was no significant effect on milk protein. Infecting the ewes had no significant effect on milk yield or composition. The HMP treatment ewes had significantly higher blood albumin levels (P=0.004), urea levels (P<0.001) and  $\beta$ -Hydroxybutyrate ( $\beta$ HB) levels (P=0.032) than the BMP treated ewes. Table 1 displays the arithmetic means with transformed means and S.E.D.s in brackets.

**Table 1** – The effect of MP and *Teladorsagia circumcincta* infection on ewe FECs, worm burdens and performance.

	Treatment				S.E.D.	Significance		
	HMP N	BMP N	HMP I	BMP I		MP	Infection	Interaction
FEC (eggs/g of faeces)	57.5 (1.44)	27.2 (1.12)	12.4 (1.06)	42.2 (1.47)	(0.283)	ns	ns	ns
Total worm burdens	1132 (2.56)	165 (1.45)	536 (2.02)	768 (1.67)	(0.545)	ns	ns	ns
Eosinophils (x10 <sup>4</sup> /l)	5.85	7.64	11.6	14.4	2.50	ns	**	ns
Milk yield (ml/day)	2251	1611	2106	1576	295.3	*	ns	ns
Milk fat (g/kg)	50.0	55.8	52.7	54.8	2.22	*	ns	ns
Milk protein (g/kg)	39.5	40.3	40.5	39.3	0.81	ns	ns	ns
Ewe weights (kg)	67.4	65.7	67.1	66.8	3.35	ns	ns	ns
Ewe condition score	1.99	2.04	2.03	1.85	0.102	ns	ns	ns
Blood albumin (g/l)	30.8	28.3	29.3	27.7	0.899	**	ns	ns
Blood urea (mmol/l)	11.2	5.16	11.9	4.95	0.498	***	ns	ns
Blood $\beta$ HB (mmol/l)	0.59	0.27	0.65	0.54	0.039	*	ns	ns
Lamb birthweights (kg)	4.08	4.20	3.97	3.88	0.286	ns	ns	ns

Key ns = non-significant (P > 0.05), \* P < 0.05, \*\* P < 0.01, \*\*\* P < 0.001

**Conclusions** Increasing the MP supply to mature dairy ewes had no effect on FEC or worm burdens. Parasite challenge resulted in an increase in eosinophil levels in ewes on both diets, which may indicate that all dietary treatments provided adequate nutrition to maintain an immune response against *T. circumcincta*. These results could suggest that protein may not be the only nutritional parameter influencing the maintenance of an immune response to parasitic infection during the periparturient period.

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# Leucocyte sub-sets and acute phase proteins are associated with productivity in Large White pigs

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**Introduction** The exposure of pigs to different pathogens compromises productivity, i.e. reduces weight gain and food intake (Balaji *et al.*, 2000; Greiner *et al.*, 2000), even in the absence of clinical disease. Infection also often causes the release of acute phase proteins and the proliferation of certain leucocyte subsets (Baumann and Gauldie, 1994; Licence and Binns, 1995). Therefore, it may be hypothesised that associations should exist between productivity and a range of immunological traits in apparently-healthy animals facing the same infectious challenge. This would be important in pigs suffering sub-clinical infections, as it would enable predictions of health status and effects of infection on performance. This study investigated such associations in apparently-healthy pigs facing unknown subclinical challenges.

**Methods** 128 Large White pigs, 62 male, 66 female, were studied from age 14 to 24 weeks. The pigs were housed together in weekly groups of 6 to 9 pigs. For each animal, cumulative food intake was recorded and live weight was recorded at 14, 18 and 24 weeks. At 18 and 24 weeks, peripheral blood samples were obtained for the measurement of (a) total and differential white blood cell counts, (b) mononuclear cell sub-sets positive for CD4, CD8, MIL-4, gamma delta, B cell and monocyte markers, and (c) alpha-1 acid glycoprotein (AGP) and haptoglobin, both of which are acute phase proteins. The associations between immune and productivity traits were quantified by multiple regression analysis using Residual Maximum Likelihood (REML). The dependent variables in each analysis were daily gain, daily food intake or food efficiency (gain/food intake) describing the whole performance test period. The independent variables in each analysis included sex and day of measurement (i.e. weekly group). Explanatory covariables were either total and differential white blood cell counts (fitted simultaneously) or the proportions of each mononuclear cell sub-set (fitted simultaneously) or acute phase proteins (fitted simultaneously). Correlations between performance and immune traits were estimated after pre-correcting each trait for the effects of sex and day of measurement.

**Results** No pigs in this study showed clinical signs of infection, yet significant relationships between performance traits and immune measurements were observed. At 24 weeks, significant relationships (shown in Table 1) were always seen for one or more of the leucocyte subset proportions, often observed for the individual acute phase proteins, but not observed for white blood cell counts. Moreover, significant relationships were always negative, i.e. an increase in the immune measurement was always associated with decreased performance. At 18 weeks, there were also negative relationships between alpha-1 acid glycoprotein and daily gain (regression coefficient  $\times 10^3 = -0.31 \pm 0.05$ ,  $p < 0.01$ ) and daily food intake (regression coefficient  $\times 10^3 = -0.78 \pm 0.15$ ,  $p < 0.01$ ), and between MIL-4 positive cells and daily gain (regression coefficient  $\times 10^3 = -6.16 \pm 2.44$ ,  $p < 0.01$ ).

**Table 1** Regression of performance traits on leucocyte sub-sets and acute phase proteins in 24-week old pigs.

Trait	significant measurements	(units)	regression coefficient $\times 10^3$	correlation (r)
Daily weight gain (kg/day)	MIL-4	(proportion)	-926 $\pm$ 307**	-0.30
	monocytes	(proportion)	-640 $\pm$ 276*	-0.29
	alpha-1 acid glycoprotein	( $\mu$ g/ml)	-0.40 $\pm$ 0.11**	-0.48
	haptoglobin	(mg/ml)	-66.7 $\pm$ 31.3*	-0.35
Daily food intake (kg/day)	B cells <sup>†</sup>	(proportion)	-15880 $\pm$ 5670**	-0.36
	alpha-1 acid glycoprotein	( $\mu$ g/ml)	-1.07 $\pm$ 0.35**	-0.38
Gain/ food intake (kg/kg)	MIL-4	(proportion)	-341 $\pm$ 81**	-0.38
	haptoglobin	(mg/ml)	-24.5 $\pm$ 9.53**	-0.34

\*\* P < 0.01, \* P < 0.05, † B cell proportion was square root transformed to reduce skewness

**Conclusion** These results indicate an association between productivity and certain immune cell types, *viz.* monocytes, B cells, MIL-4 positive cells and acute phase proteins (alpha-1 acid glycoprotein (AGP) and haptoglobin), in apparently-healthy pigs. Sub-clinical infection may be one cause for this association. If so, then these immune measurements may be predictive of pig health status and performance.

**Acknowledgements** This work was funded, through LINK SLP, by the Generalised Immunity Pig Consortium and the Department of Environment, Food and Rural Affairs (DEFRA), and the BBSRC.

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## Changes in the mechanical properties and the lesion score of the sole horn in first lactation heifers

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**Introduction** The start of the lactation period is related to an increase in the number and severity of claw horn lesions on dairy cows and heifers (Offer *et al.*, 2000). Heifers have important physiological differences, presenting a different pattern of lesion formation when compared to cows (Offer *et al.*, 2000). The rearing of heifers, their growth rate, feeding and the occurrence of foot problems have an influence over the health of their feet when the animals get older (Thomas *et al.*, 1999). The objective of this experiment was to study the pattern of lesion formation of the sole horn in the pre- and postpartum period of first calving heifers and to compare it to mechanical tests.

**Materials and Methods** Mechanical tests were performed on samples of hoof horn of the sole taken from 20 heifers at two months before calving and at 50, 100 and 150 days after calving. All claws were scored for the level of haemorrhage and ulceration of the sole horn before calving and 100 days after calving. The heifers were kept at pasture and joined the lactating cow group one month before calving. After calving the heifers were kept in a straw yard in a separate group. Samples for the mechanical tests were collected of all claws and analysed on a texture analyser for puncture resistance using a P/2N needle probe. Five puncture tests were completed on the sole (area 5) of each claw. The data was analysed by ANOVA – GLM (Minitab 12.0), using cow and claw as fixed effects and thickness as a covariant when puncture tests were analysed. The effect of collection period on puncture force was tested by regression analysis.

**Results** The mean values for puncture force of the sole horn and lesion score of the front and hind claws, are presented in Table 1. The puncture force of the sole horn was significantly greater in the front claws when compared to the hind claws in all collection periods ( $p < 0.01$ ), but no significant difference was obtained between the inner and outer claws of front and hind feet. The puncture force increased from the pre-calving period until 100 days post calving ( $force = 494.41 + 3440.1 \text{ period}$ ,  $R^2 = 0.466$ ). The lesion score of the claw horn increased from the pre-calving period until 100 days post calving ( $p < 0.001$ ). No difference in the lesion score was observed between claws in the pre calving period. At 100 days *post partum* the lesion score was significantly greater in the hind claws when compared to the front claws ( $p < 0.001$ ) and the increase in the lesion score was also significantly greater in the hind claws when compared to the front claws ( $p < 0.001$ ). In the hind feet the outer claws presented a significantly ( $p < 0.05$ ) greater lesion score when compared to the inner claws in both periods. In the front feet the lesion score was significantly higher ( $p < 0.05$ ) in the outer claws before parturition and in the inner claws ( $p < 0.01$ ) after parturition.

**Table 1** Puncture force (N) of the sole horn and lesion score of front and hind claws 1 month pre-calving and 100 days after calving in first lactation heifers

Claws	front	hind	sem	<i>p</i>
Lesion score pre-calving	71.09	75.05	6.21	ns
Lesion score 100 days after calving	149.32	223.72	9.00	0.001
Increase in lesion score	78.23	148.67	10.59	0.001
Puncture force pre-calving	8.24	7.41	0.46	0.01
Puncture force 100 days after calving	11.06	10.32	0.24	0.05

**Conclusions** The increase in lesion score after calving was reported before in heifers and cows (Offer *et al.*, 2000). The higher increase in lesion score of the hind claws is probably related to the conformation of the udder at parturition that forces the cow to have a wider stance, causing increased weight bearing in the outer hind claws (Toussaint-Raven, 1985). In heifers differences in the lesion formation are probably related to the rearing and management. The increased resistance to puncture in front claws when compared to hind claws, is likely to be related to the different pressure distribution in these claws, predisposing the hind claw to suffer lesions through contusion. The increase in the puncture resistance after calving was not expected. In cows housed in cubicles lactation and housing were related to a decrease in puncture resistance of the sole horn (Winkler *et al.*, 2002). These findings demonstrate the importance of studying the influence that the management and rearing of heifers have over the structure of the hoof horn.

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# Effect of mixing piglets prior to weaning on immune responses of piglets

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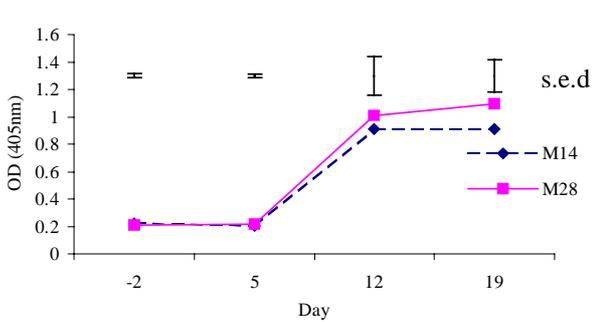
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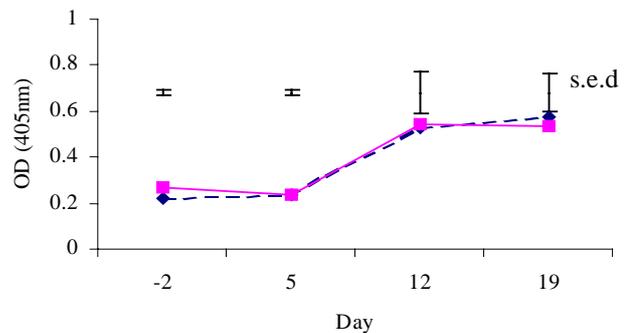
**Introduction** Mixing piglets pre-weaning at 14 days of age has been shown to improve post-weaning performance and reduce skin lesions caused by fighting without any detrimental effects on pre-weaning performance and behaviour (Allen *et al.*, 2000). The stress associated with weaning has been shown to alter immune function of piglets and increase their susceptibility to infections. The aim of this experiment was to assess the effect of mixing piglets pre-weaning on their humoral and cell-mediated immune responses post-weaning.

**Material and methods** Twelve PIC Camborough 15 (Large White x (Landrace x Duroc)) sows and their litters kept in conventional farrowing crates were randomly allocated to either a control group (C) which were mixed at weaning (28 days of age) or where piglets were mixed at 14 days of age (treatment M). 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. Creep feed was available from day 17, weaning took place on day 28 and piglets were relocated to fully slatted accommodation. Sixteen piglets (four randomly selected piglets per litter) were immunised with keyhole limpet haemocyanin (KLH) at weaning (Day 0). Class and subclass anti-KLH antibody response was measured by an ELISA method and cellular immune responses were assessed by *in vitro* lymphocyte blastogenic responses to the mitogen concanavalin A. Skewed data was log transformed and covariate ANOVA was carried out using Day -2 as the covariate.

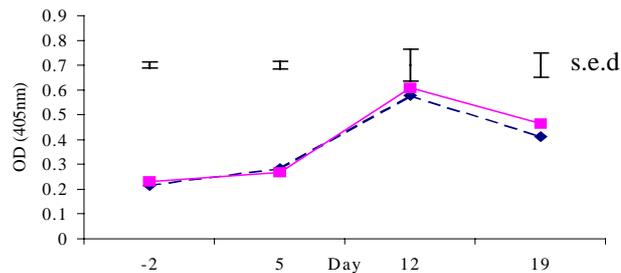
**Results** A typical primary immune response to a novel antigen was observed in all piglets' post-immunisation. Piglets mixed at weaning (C) had significantly higher anti-KLH IgG<sub>1</sub>, IgM and IgA levels compared with piglets mixed prior to weaning (M) (P<0.001 for IgG<sub>1</sub> & IgM and P<0.01 for IgA respectively) on day 12. (Figures 1-4 - raw data presented).



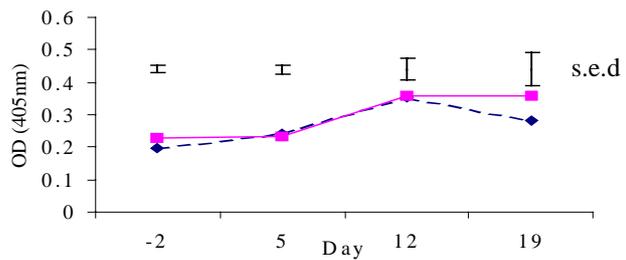
**Figure 1:** Effect of mixing on anti-KLH IgG<sub>1</sub> response post-weaning



**Figure 2:** Effect of mixing on anti-KLH IgG<sub>2</sub> response post-weaning



**Figure 3:** Effect of mixing on anti-KLH IgM response post-weaning



**Figure 4:** Effect of mixing on anti-KLH IgA response post-weaning

Piglets mixed prior to weaning (M) had a significantly higher lymphocyte blastogenesis stimulation index on day 5 compared with piglets conventionally mixed at weaning (C) (6.65 versus 4.61 mean log<sub>e</sub> CPM of <sup>3</sup>H-thymidine uptake; s.e.d. 0.797, p>0.05). There was no significant effect of mixing on lymphocyte blastogenesis on day 19.

**Conclusions** Elevated humoral responses and reduced lymphocyte blastogenic responses in piglets mixed at weaning supports work by Mackenzie *et al.* (1993) where the similar trends were reported in weaned calves compared with calves remaining on their dam. Therefore, this work supports the hypothesis that mixing pre-weaning may be beneficial to the piglet post-weaning.

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## Preferential associations between group-housed growing pigs

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**Introduction** Evidence suggests that pigs prefer to associate with their mother and littermates over other group members (e.g. Newberry & Wood-Gush, 1986) and with pigs introduced with them into an established group over resident pigs (Durrell *et al.*, 2000). Few studies, however, have examined whether long-term preferential associations or 'friendships' are formed between pairs of pigs within a group. Those studies that have been carried out have either involved observations carried out over extremely limited time periods (e.g. Stookey & Gonyou, 1998) or have simply identified pairs that spend the most time together instead of examining statistically whether some pairs associate significantly more than others (Newberry & Wood-Gush, 1986). The aim of this investigation was to determine whether pairs of pigs form preferential associations, based on statistical analyses of long-term lying partner preferences.

**Materials and Methods** Thirty-three Large White x Landrace pigs from 16 litters were housed together from 4-weeks of age. At 10-weeks they were split into two groups of 16 and 17 pigs and each introduced into a 3.05m x 3.66m observation pen (1<sup>st</sup> pen). At 17-weeks the two groups swapped pens (2<sup>nd</sup> pen). The two pens were identical mirror images of each other on opposite sides of an aisle. Six hourly video recordings were obtained three days a week over 3-weeks in each pen, commencing one week after the pigs' introduction. From these recordings, mean changes of lying positions and lying partners were estimated at 25 and 20 minutes respectively. From these recordings, the lying patterns of pigs were recorded using scan sampling at 15-minute intervals, where the identities of all pairs lying together and all pigs lying alone were recorded. Fifteen-minute intervals were deemed appropriate, since observations showed that, on average, these pigs changed lying positions every 25 minutes and lying partners every 19 minutes. The data were analysed using SOCPROG1.3 (Whitehead 1999). Half-weight association indices for each pair were displayed in an association matrix and separate matrices were drawn up for each group (groups 1 and 2) within each pen (1<sup>st</sup> and 2<sup>nd</sup> pen) and during each observation week (weeks 1 to 6). Two hundred and fifty thousand permutations were carried out on each observed association matrix using the Monte Carlo method described by Bejder *et al.* (1998) and Manly (1995) and for each permutation, a new random association matrix was formulated. If the standard deviation for an observed association matrix was greater than 95% of those of its permuted association matrices this showed that a group contained dyads with significantly high association indices. If the association index for a dyad was greater than 95% of those of its permuted indices then this showed that the dyad had a significantly high association index. Dyads with a significantly high index for at least two weeks in one pen were classed as either showing a short-term preferential association or a shared lying location preference. Dyads with a significantly high index for at least two weeks in both pens were classed as showing a long-term preferential association.

**Results** The existence of preferential associations were identified, since the standard deviations for the observed HWI means were significantly higher than for the randomly permuted HWI means for group 1 in pen 1 (observed HWI: 0.06 ± 0.029, permuted HWI: 0.06 ± 0.020,  $P < 0.001$ ) and pen 2 (observed HWI: 0.05 ± 0.028, permuted HWI: 0.05 ± 0.019,  $P < 0.001$ ) and for group 2 in pen 1 (observed HWI: 0.06 ± 0.038, permuted HWI: 0.06 ± 0.026,  $P < 0.001$ ) and pen 2 (observed HWI: 0.05 ± 0.035, permuted HWI: 0.05 ± 0.023,  $P < 0.001$ ). Of the 33 pigs observed, 20 formed preferential associations with one or more pigs in their group. However, this amounted to just 13 dyads (out of a possible 256 dyads. This included 12 dyads for at least 2-weeks in one pen (over 3-week period - observed HWI: 0.06-0.26, SD: 0.028-0.038  $P < 0.05$ -0.001) and just one dyad for at least 2 weeks in both the 1<sup>st</sup> (over 3-week period - observed HWI: 0.15, SD: 0.038,  $P < 0.001$ ) and 2nd pens (over 3-week period - observed HWI: 0.15, SD: 0.035,  $P < 0.001$ ).

**Conclusions** The findings suggest that although pigs do appear capable of forming preferential associations as measured by their lying patterns, it is unclear whether such associations are widespread or important to pigs, since only one dyad showed clear evidence of a long-term preferential association. Twelve other dyads were identified, comprising 20 of the remaining 31 pigs. However, these dyads did not show preferential associations in both pens, which either suggests they formed short-term associations only or that they shared the same preferred lying location within their pen.

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# Stonechewing in pigs is influenced by feed level and by previous stonechewing experience

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**Introduction.** Adult pigs maintained outdoors spend a very high proportion of their time chewing stones. The activity is persistent, taking up 30-50% of the 6 h after feeding, and highly repetitive in its pattern of component activities; it is reminiscent of the stereotypies (bar-biting, vacuum chewing, rooting at floors and foot-scraping) in stall- and cubicle-housed sows indoors. Consistent with this hypothetical parallel, the amount of stonechewing in outdoor sows in different environments increased systematically with impoverishment in the habitat (Horrell and A'Ness, 1999). Two factors that influence the incidence of oral stereotypies are feed restriction (Appleby and Lawrence, 1987) and a past history of stereotypical behaviour (Lawrence et al., 1991). This project aimed to determine whether these factors have an impact on the incidence of stonechewing.

**Materials and method.** Data were collected in 2 groups of sows of the same strain, in their third or higher pregnancy:-  
 1. Permanently housed indoors in straw yards except for parturition and lactation in farrowing pens ('IN': N = 8)  
 2. Moved as breeding gilts shortly before their first farrowing to, arable paddocks with straw floored ark, and thereafter permanently maintained outdoors ('OUT': N = 9)

Two weeks before observations commenced, each group was moved to straw-bedded housing, with a 8.4 x 2.4 m outer covered yard and two enclosed 4.2 x 1.5 m lying areas. Normal feed level of 2.5 kg/d (ME: 13 MJ) delivered to groups was modified throughout 5-d periods to one of two levels: 1.5 kg/d or 3.5 kg/d. The schedule over each 5-d period consisted of 3 d habituation to the new feed level followed by 2 d, when observations were made for 6 h/d, starting 30 min before feed delivery. Each group experienced a single sequence of treatments involving two periods at each feed level, with orders counterbalanced to ensure comparability between both feed level and normal maintenance condition (IN/OUT). During observations, the number of sows engaged in each of a wide range of behaviours was counted every 3 mins. From these data, the mean probability of a pig being engaged in each activity at any instant was calculated, from which the mean time (in min/h) that a pig spent engaged in an activity was inferred. Data were submitted to 2-way analyses of variance, with repeated measures on feed level, for each behaviour. The low frequency of 'abnormal behaviour' occurring at sampling instants necessitated the use of a chi square analysis on the actual number of events.

**Results.** The time spent stonechewing in outdoor pigs was consistently more than twice that of IN (Table 1). IN pigs, on the other hand, spent more time than OUT pigs chewing straw at both feed levels, and more time rooting. Feed restriction increased stonechewing, and to a greater extent in OUT than IN pigs (interaction  $p < .05$ ). Similarly, the incidence of straw-chewing was raised by feed restriction. Abnormal behaviour (vacuum chewing, persistent scraping at the ground with foreleg, sustained biting and/or licking at bars, and belly-nosing), an independent indicator of welfare, was rare in OUT pigs, but consistently more frequent in IN pigs, and increased in the latter with feed restriction (chi sq.:  $p < .05$ )

**Table 1.** Mean time/hour (mins/hr) with S.E., spent stonechewing, rooting and chewing straw by sows in each treatment condition; total number of incidents observed at sampling instants for 'abnormal behaviour'. (Means separated by \*\*\* for  $p < .001$ , \*\* for  $p < .01$  or \* for  $p < .05$ , are statistically significant)

Activity:	Stonechewing		Rooting		Chewing straw		Abnormal behaviour	
	OUT	IN	OUT	IN	OUT	IN	OUT	IN
<b>High feed</b>	25.28 (2.49)***	9.29 (1.26)	1.94 (0.76)*	5.05 (1.09)	2.26 (1.15)**	7.01 (0.88)	4	11
<b>Low feed</b>	37.12 (2.82)***	13.87 (1.65)	2.47 (0.82)	5.81 (1.75)	2.55 (0.85)***	13.89 (1.70)	1	35

**Conclusions.** The facts that a reduction in feed ration and prior experience of stonechewing (which just about all adult pigs in outdoor production units engage in) both increased the incidence of and time spent in stonechewing is consistent with the hypothesis that it is an oral stereotypy with the same origin as the oral stereotypies seen in sows individually housed in stalls and cubicles indoors. Further, the greater level of rooting and straw-chewing in IN, with the latter also responsive to feed restriction, at the same time as a lower level of stonechewing, suggests that these behaviours may be substitutable to an extent, IN pigs using rooting and straw-chewing where OUT pigs may be more inclined to stonechew. Finally, the higher levels of other abnormal behaviour in IN pigs, compared to OUT, is consistent with the possibility that OUT pigs' greater levels of stonechewing may somehow partially relieve the impact on the welfare of OUT pigs in these conditions.

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**Acknowledgements.** We thank De Montfort University for provision of pig unit facilities and the Universities Federation for Animal Welfare for financial support for this project.

## Alternatives to nose-ringing in outdoor sows: the provision of root crops

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**Introduction** Nose rings have been proven to reduce the occurrence of paddock rooting behaviour, however, the practice has been questioned on ethical grounds. The aim of this experiment was to identify a suitable alternative. Previous attempts to reduce paddock damage by dietary means have succeeded in redirecting rooting behaviour (Bornett *et al.*, 2002) or at reducing the overall frequency of paddock rooting behaviour (Braund *et al.*, 1998) but neither succeeded in reducing paddock damage. It is hypothesised that whilst the experiment of Bornett *et al.* (2002) fulfilled the sow's desire to forage, and the experiment of Braund *et al.* (1998) fulfilled the sow's need to feel satiated; neither fulfilled both criteria simultaneously. In this experiment, both of these needs were considered and it was therefore hypothesised that paddock damage would be reduced.

**Materials and Methods** 16 pregnant multiparous sows were housed in groups of four and randomly allocated to one of four dietary treatments in a 4x4 Latin square design with a 2 week period. Treatment A acted as a control and had no sacrificial rooting area in the paddock; Treatments B,C and D were all provided with a rooting area consisting of a 15 x 2m strip of ploughed land. Sows in Treatment B received 5kg of swedes/sow spread over the surface of the paddock in addition to their daily concentrate feed ration. Treatment C received 5kg of swedes/sow buried in the rooting area, again in addition to their daily ration of concentrate feed. Sows on treatment D also received 5kg swedes/sows buried in the rooting area, but in this treatment the concentrate ration was reduced by 0.5kg/sow to provide a complete diet isoenergetic to that offered to the control sows. Behavioural observations were made on two days of the week over four, one hour periods. Sow groups were rotated between paddocks, such that the same treatment was always applied in any individual paddock to allow cumulative effects on paddock condition to be quantified. Pasture damage was assessed weekly using a standard quadrat sampling pattern. Data were analysed using an analysis of variance.

**Results** Sows that received swedes spent significantly less time rooting the paddock when compared to the control sows (A=8.66%, B=5.65%, C=6.64%, D=4.72% , P<0.001). The paddock housing sows receiving the isoenergetic diet (containing swedes) had highest levels of vegetation cover at the end of the eight week period (55% cover) although this was not significantly different from the other three treatments (control=31%, paddock=38%, buried=30%).

**Table 1** The effect of dietary treatment on the percentage of observation time spent in different behaviours by outdoor sows

	Control(A)	Paddock(B)	Buried (C)	Isoenergetic (D)	sed	P
Rooting Paddock	8.66 <sup>a</sup>	5.65 <sup>b</sup>	6.64 <sup>b</sup>	4.72 <sup>b</sup>	1.413	***
Rooting Sacrificial Area	-	2.32 <sup>a</sup>	7.03 <sup>b</sup>	8.14 <sup>b</sup>	1.669	*
Total Rooting	8.66 <sup>a</sup>	7.97 <sup>a</sup>	13.67 <sup>b</sup>	12.86 <sup>b</sup>	1.913	*
Eating Swede Paddock	-	9.03 <sup>a</sup>	2.54 <sup>b</sup>	3.76 <sup>b</sup>	1.943	*
Eating Swede Sac. Area	-	0.06 <sup>a</sup>	2.23 <sup>b</sup>	4.00 <sup>b</sup>	1.018	*
Eating Swede total	-	9.09	4.77	7.76	2.825	ns
Grazing	42.22 <sup>a</sup>	26.69 <sup>b</sup>	25.07 <sup>b</sup>	33.47 <sup>ab</sup>	4.352	**
Total Foraging	52.33	44.62	44.39	54.63	5.369	ns

**Conclusions** The provision of the sacrificial area containing swedes significantly reduced paddock rooting behaviour but alone is not able to radically reduce paddock damage. Therefore this strategy cannot be recommended as a sole commercial alternative to nose-ringing sows.

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**Acknowledgements** The authors would like to thank the Royal Society for the Prevention of Cruelty to Animals for funding this project

## Preference of pigs for illuminance

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**Introduction** Pigs housed under artificial lighting currently experience a wide range of illuminances and photoperiods, which may be more appropriate to the visual capabilities and needs of stockpersons rather than pigs. Pigs and wild boar can show nocturnal, diurnal and crepuscular activity patterns, suggesting that their visual system may function well under a wide range of light levels, unlike humans. Inappropriate lighting affects many aspects of an animal's physiology, anatomy and behaviour and may compromise welfare. This experiment was designed to investigate the preference of juvenile pigs to occupy and conduct certain behaviours in different illuminances, and gain some indication of their preferred photoperiod.

**Material and Methods** Eight weaned gilts (Large White x Landrace x Duroc) were housed in a light-proofed indoor environment. An illuminance gradient along the pen from 400 lux to <1 lux was provided continuously and reversed every two days. The feeder was also moved between the middle and ends of the pen. At seven weeks of age, the gilts were divided into two groups of four and introduced into an annular preference chamber. Each group of four pigs could choose to occupy any of four compartments within the chamber; the compartments differed only in illuminance and were equally provided with food, water and shavings. Each compartment received one of four illuminances, minimum (<4 lux), 4 lux, 40 lux and 400 lux. The pigs occupied the chamber for two acclimatisation days followed by eight test days. Every two days, the pigs were removed while the chamber was cleaned and the illuminances changed in each compartment according to a balanced randomised pattern. Lighting was provided by incandescent bulbs placed over the otherwise lightproof lids to the compartments, and dimming was principally achieved using layers of frosted gel filter paper, with fine adjustment from dimmer switches; this maintained similar spectral output regardless of the compartment's illuminance. Activity in the chamber was video recorded and analysed by scan sampling all pigs at intervals of six minutes for the central 24 hours of each two-day occupation period. The study was repeated with the initial batch of eight pigs at 11 weeks of age, and replicated with another batch of eight pigs at 7 and 11 weeks of age.

**Results and Discussion** The analysed data are from the two replicates of the first batch at ages 7 and 11 weeks but due to a technical fault, only the two replicates of the second batch at 11 weeks - which affected the intended, combined analysis. Instead, the data were logarithmically transformed and analysed by two general ANOVAs, one for batch 1 at 7 weeks, another for both batches at 11 weeks, with each group of four pigs treated as a unit.

**Table 1** Occupancy and inactivity of pigs under different illuminances, showing backtransformed means, with transformed means and their corresponding s.e.ds in brackets

Batch	Age (wks)	Occupancy per 24 hours (h)					Time inactive per 24 hours (h)				
		<4 lux	4 lux	40 lux	400 lux	(s.e.d)	<4 lux	4 lux	40 lux	400 lux	(s.e.d)
1	7	10.3 (5.74)	7.5 (5.43)	3.33 (4.62)	2.9 (4.47)	(0.474)	7.9 (5.50)	5.8 (5.19)	1.8 (4.03)	1.2 (3.64)	(0.428)
1 & 2	11	8.6 (5.74)	5.6 (5.30)	6.2 (5.40)	3.7 (4.90)	(0.191)	6.6 (5.51)	3.7 (4.93)	4.9 (5.11)	2.3 (4.45)	(0.207)

In general, the two dimmer illuminances were preferred to the brighter ones. Occupancy of <4 lux was always significantly greater than use of 40 and 400 lux ( $P < 0.05$ , d.f. = 69). The pigs' main occupation was lying inactive (approx 19.5 h per day), for which 4 lux or less were significantly preferred ( $P < 0.05$ , d.f. = 271). Eating was the only active behaviour for which the time budget was affected in a consistent and significant way by illuminance. More eating was observed under <4 lux than 400 lux for all batches analysed (28.3 v 13.5 mins (transformed data 2.74 v 2.07, s.e.d.= 0.20, d.f. = 1055)  $P < 0.05$ ). There was no clear pattern of illuminance use over a 24 hour period, thus activity pattern itself was a more useful indicator of daily rhythm. The pigs were least active between 00:00 and 07:00, and consistently more active from 07:00 to 18:00. A distinct peak in activity occurred between 09:00 and 10:00, but this coincided with general activity in the building and so may not be a true indication of the pigs' innate activity pattern. Overall, a diurnal pattern of activity was seen, however effects of external "Zeitgeber" cannot be ruled out.

**Conclusions** Illuminances of  $\leq 4$  lux were preferred by juvenile gilts especially when resting or sleeping, which was their main occupation. These results suggest that spatial and/or temporal provision of low illuminance in pig housing is consistent with their preferences and may improve welfare. This finding contradicts EC recommendations which state that illuminance of pig housing should be a minimum of 40 lux provided for eight hours per day (EC 2001).

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**Acknowledgements** The authors are grateful to the BBSRC and Freedom Food for funding this work through a CASE scholarship

# Influence of social status on the welfare of growing pigs reared in barren and enriched environments

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**Introduction** Evidence suggests that low social status contributes to reduced welfare in pigs (O'Connell et al., in press). It may be possible to reduce this effect by rearing pigs in enriched environments, which has previously been shown to have welfare benefits (Beattie et al., 2000). The aim of this study was to assess the effect of social status on the welfare of pigs reared in barren or enriched environments.

**Materials and methods** 128 pigs were used in a 2 x 2 factorial design experiment with two replicates. The factors were social status (high or low) and rearing environment (barren or enriched). In each replicate, four groups of pigs were reared from birth to slaughter at 21 weeks of age in either barren or enriched environments. Barren environments had slatted floors and recommended space allowances, whereas enriched environments contained substrates and, after 6 weeks of age, additional space. Pigs were housed as litter groups until 8 weeks of age. At this stage, the four litter groups in each environmental treatment (in each replicate) were mixed to form four groups of eight pigs which contained one boar and one gilt from each litter. All groups were mixed in standard barren mixing pens where they remained for 24 hours. Aggressive and submissive behaviours were recorded continuously for 8 hours after mixing. High or low social status was assigned to each pig on the basis of relative success in aggressive interactions. Approximately half the pigs in each group were assigned high social status and half were assigned low social status. Injuries to the ears, and to the head and neck area were assessed each week from 8 to 21 weeks of age. Pigs were exposed to two group food competition tests at 10 weeks of age. Each test lasted for 15 minutes and took place after 18 hours of food deprivation. A dominance index value was calculated for each pig by expressing the number of individuals the pig displaced from the feeder during the test as a percentage of the number of individuals the pig displaced plus the number of individuals which displaced the pig from the feeder. Plasma cortisol responses to both the food competition test plus the period of feed restriction were assessed in both tests. In addition, each pig was individually exposed to a novel object in a novel environment for a 7 minute period at 11 weeks of age, and behavioural and plasma cortisol responses recorded. Data were analysed by analysis of variance using Genstat 5.

**Results** Effects of social status on selected parameters are listed in Table 1. Pigs with low social status showed greater levels of injury to the head and neck area, and also to the ears than pigs with high social status ( $P<0.01$ ). Pigs with low social status had a reduced relative ability to displace penmates from the feeder in both food competition tests and therefore had lower dominance index values ( $P<0.06$  in test 1, and  $P<0.001$  in test 2). Pigs with low social status also contacted the novel object less frequently during the novel environment test than pigs with high social status ( $P<0.05$ ).

**Table 1** Influence of social status (SS) on injury and behavioural parameters in growing pigs

Parameter	High SS	Low SS	s.e.m.	Significance
<i>Injury score:</i>				
Head and neck	0.054	0.102	0.0113	**
Ears	0.122	0.178	0.0127	**
<i>Food competition test (average tests 1 and 2)</i>				
Dominance index value (%)	37.9	25.4	2.62	**
<i>Novel pen test</i>				
Contact with novel object ( $\text{min}^{-1}$ )	0.21	0.13	0.027	*

In terms of interactive effects, in barren environments low social status pigs (LSS) showed greater latencies to contact the novel object in the novel environment test than high social status pigs (HSS) (HSS 80.9, LSS 141.1, s.e.m. 12.51 s,  $P<0.01$ ), whereas this effect was not shown in enriched environments (HSS 135.3, LSS 110.3, s.e.m. 12.51 s,  $P>0.05$ ). In addition, in barren environments LSS pigs showed greater plasma cortisol responses in the first food competition test than HSS pigs (HSS 21.2, LSS 55.8, s.e.m. 11.66 nmol/l,  $P<0.05$ ), whereas this effect was not shown in enriched environments (HSS 26.1, LSS 9.5, s.e.m. 11.66 nmol/l,  $P>0.05$ ). Rearing environment did not significantly affect either of these parameters ( $P>0.05$ ).

**Conclusions** Low social status had a negative effect on welfare as it resulted in increased injury and a reduced ability to gain access to resources. In addition, pigs with low social status appeared more fearful by showing greater avoidance of a novel object. Environmental enrichment appeared to improve the welfare of pigs with low social status by reducing stress associated with the food competition test and fearfulness associated with the novel environment test.

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## Optimising the fatty acid composition of beef muscle

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**Introduction** The Department of Health (1994) recommended people to reduce their intake of total fat and saturated fat and increase that of *n*-3 polyunsaturated fatty acids (PUFA) to 200 mg/d. The ratio of PUFA:saturated fatty acids (P:S ratio) in the total diet should be >0.4 with an *n*-6:*n*-3 ratio of <4. Since fish consumption, a major source of dietary long-chain *n*-3 PUFA, is low in the UK, research has focused on improving the fatty acid balance of other meats. The objective of this paper is to summarise studies by IGER and the University of Bristol on manipulating fatty acid composition of beef.

**Materials and methods** In an initial study the fatty acid content and composition of retail beef was assessed in fifty beef sirloin steaks (Enser *et al.*, 1996). Subsequent studies examined the effects of different sources of dietary *n*-3 PUFA on fatty acid composition of *m.longissimus* muscle (Scollan *et al.*, 2001). Charolais steers were fed *ad libitum* grass silage plus concentrates (60:40 forage:concentrate DM ratio) containing either Megalac (rich in 16:0 from palm oil, Volac Ltd, Royston), lightly bruised whole linseed (rich in  $\alpha$ -linolenic acid, 18:3*n*-3), fish oil (rich in 20:5*n*-3, EPA and 22:6*n*-3, DHA) or a ruminally protected lipid supplement (PLS; consisting of soya beans, sunflower oil and linseed with 18:2*n*-6:18:3*n*-3 ratio of 2:1; Scollan *et al.*, 2002a). The effects of raising Simmental steers from 270 to 650 kg on a forage only diet rich in 18:3*n*-3 consisting of grass and white clover was assessed by Scollan *et al.* (2002b). Total saturated fatty acids (SFA) were calculated as sum of 14:0 + 16:0 + 18:0. Total ( ) PUFA = sum of all *n*-6 and *n*-3 PUFA. Total *n*-3 PUFA = 18:3*n*-3 + 20:4*n*-3 + 20:5*n*-3 + 22:5*n*-3 and 22:6*n*-3. P:S and *n*-6:*n*-3 were calculated according to Scollan *et al.* (2001a).

**Results** All the beef analysed was lean with intramuscular fat ranging between 26 and 44 g/kg (see Table). Linseed relative to megalac not only doubled the levels of 18:3*n*-3 but also enhanced EPA. Fish oil doubled the proportion of EPA and DHA. The P:S was unchanged by feeding linseed or fish oil but *n*-6:*n*-3 ratio was markedly improved. Feeding PLS reduced total intramuscular fat and SFA and increased PUFA content. Grass/white clover resulted in beef with high total PUFA, rich in *n*-3, contributing to a low *n*-6:*n*-3 ratio.

**Table 1** Effect of diet on the fatty acid composition of longissimus dorsi muscle (mg/100 g muscle)

	Total fat	18:2 <i>n</i> -6	18:3 <i>n</i> -3	EPA	DHA	SFA	PUFA	<i>n</i> -3 PUFA	P:S	<i>n</i> -6: <i>n</i> -3
Retail beef <sup>a</sup>	3835	89	26	10	1.6	1572	126	57	0.08	2.20
Megalac <sup>b</sup>	3359	78	20	10	2.5	1562	162	54	0.07	2.00
Linseed <sup>b</sup>	3618	69	38	15	2.7	1546	175	81	0.07	1.19
Fish oil <sup>b</sup>	4400	63	27	24	5.3	2089	187	80	0.05	0.91
Protected lipid <sup>c</sup>	2604	243	50	10	2.0	1064	361	77	0.27	3.59
Grass/white clover <sup>d</sup>	3411	94	66	27	3.6	1369	267	131	0.16	1.51

<sup>a</sup>Enser *et al.* (1996); <sup>b</sup>Scollan *et al.* (2001), <sup>c</sup>Scollan *et al.* (2002a) and <sup>d</sup>Scollan *et al.* (2002b)

**Conclusions** Beef muscle is a low fat food (< 5% fat). Retail beef contains significant quantities of *n*-3 PUFA and these may be further increased by feeding linseed or fish oil. Diets containing only forage and fed over a long period result in high PUFA meat. Ruminally protected lipid supplements offer the potential to reduce intramuscular fat content, increase PUFA deposition resulting in beef with a good P:S ratio. Assuming that the average daily consumption of beef is 100 g/d (Enser *et al.*, 1996) and recommended intake of *n*-3 PUFA is 100-200 mg/d (Department of Health, 1994) it is evident beef has an important role in providing *n*-3 PUFA in the human diet.

**Acknowledgements** This work was supported by Department for Environment, Food and Rural Affairs, ABN Limited, International Fish Oil and Meal Manufacturers Association, Tesco Stores Limited, Pedigree Petfoods, Rarebreeds Survival Trust and Roche Products Limited.

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# Effects of product type and fatty acid composition on shelf life of nutritionally modified beef

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**Introduction** Ruminant meat products have a relatively saturated fatty acid profile so lipid oxidation during the postmortem period, which could lead to off-odours and flavours and trigger undesirable colour changes, is usually minor. However, attempts to increase levels of PUFA in meat for health reasons could be compromised by increased lipid oxidation. This paper is a summary of recently completed work between University of Bristol and IGER on the links between fatty acid composition and oxidative stability in beef and seeks to draw general conclusions.

**Materials and Methods** The information is taken from 3 trials. In the first (Scollan *et al.* 2001), steers were fed a silage: concentrate diet, the concentrate containing 60g/kg lipid provided by megalac (saturated control), bruised linseed (source of 18:3 n-3), fish oil (source of 20:5 n-3 EPA and 22:6 n-3 DHA) or a 50:50 combination of linseed and fish oil. In the second (Scollan *et al.* 2002a), a protected lipid supplement (PLS, 18:2 to 18:3 = 2:1), was fed as part of a silage/concentrate diet, the concentrate containing 40g/kg as megalac, PLS or 50:50 mega:PLS. In trials 1 and 2, vitamin E was added to the concentrate at 340mg/kg. In the 3rd trial (Scollan *et al.* 2002b), steers were given grass, grass + white clover or grass + red clover diets. 24hr after slaughter in Experiment 1, forequarter muscles were minced, packed in a modified atmosphere (O<sub>2</sub>:CO<sub>2</sub> 75:25) and displayed under retail conditions. After 10d, lipid oxidation was assessed as thiobarbituric acid reacting substances (TBARS) and colour saturation (vividness of colour) was measured using a Minolta chromameter. Values of TBARS <2.0 and saturation >18 are acceptable to consumers. In Experiments 2 and 3, the same measurements were made, but on *longissimus* steaks, also packed in a modified atmosphere after 10d ageing at 1°C. Fatty acid composition was measured as described by Scollan *et al.* (2001).

**Results** In trial 1, the highest lipid oxidation and poorest colour retention was in cattle fed fish oil although the concentration of EPA was lower than in the loin of cattle fed grass diets in trial 3 (table 1). Clearly, processing into burgers was a major factor in the reduced shelf life. Greater utilisation of vitamin E had also occurred. In trial 2, the high PUFA content of PLS produced unacceptable shelf life. The results for TBARS and vitamin E are shown in Figure 1. In trial 3, high levels of 18:3 n-3 and EPA obtained in meat after grass feeding were associated with good shelf life and vitamin E concentrations were above 3.0 mg/kg threshold found by our group to be necessary for protecting PUFA in loin steaks. Vitamin E reached higher concentrations in the forequarter muscles than in *longissimus*.

Table 1. Fatty acid composition and shelf life parameters

	g/100g total fatty acids			TBARS <sup>A</sup>	Sat <sup>B</sup>	Vit E <sup>C</sup>
	18:2	18:3	EPA			
Megalac	2.6	0.6 <sup>a</sup>	0.3 <sup>a</sup>	0.6 <sup>a</sup>	20 <sup>a</sup>	6.9 <sup>a</sup>
Linseed	2.3	1.2 <sup>c</sup>	0.5 <sup>a</sup>	1.1 <sup>a</sup>	18 <sup>a</sup>	6.5 <sup>a</sup>
Fish oil	1.6	0.6 <sup>a</sup>	0.6 <sup>b</sup>	3.6 <sup>b</sup>	16 <sup>b</sup>	5.7 <sup>b</sup>
Linseed/fish	2.0	0.9 <sup>b</sup>	0.5 <sup>a</sup>	1.3 <sup>a</sup>	18 <sup>a</sup>	6.4 <sup>a</sup>
Megalac	3.0	0.7 <sup>a</sup>	0.3	0.5 <sup>a</sup>	20 <sup>a</sup>	4.5 <sup>a</sup>
Mega + PLS	6.3	1.4 <sup>b</sup>	0.3	1.9 <sup>b</sup>	18 <sup>b</sup>	3.7 <sup>b</sup>
PLS	9.3	2.0 <sup>c</sup>	0.4	3.0 <sup>c</sup>	16 <sup>a</sup>	3.8 <sup>b</sup>
Grass	2.6	2.0	1.1	0.7	18	4.0
Red clover	3.4	2.5	1.0	0.9	18	4.1
White Clover	3.1	2.2	1.0	0.6	18	3.5

<sup>A</sup>mg MDA/kg <sup>B</sup>Colour saturation units <sup>C</sup>mg/kg

<sup>abc</sup> Values in columns for a given trial with different superscripts are significantly different (P<0.05)

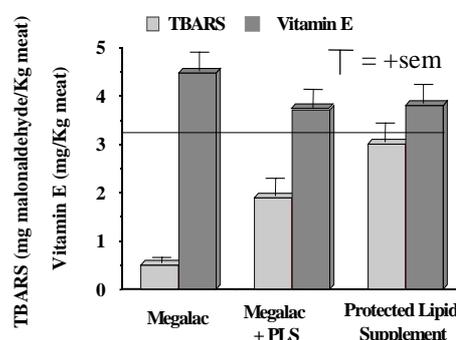


Fig 1. TBARS and vitamin E in trial 2. PLS = Protected Lipid Supplement (18:2 to 18:3 = 2:1)

**Conclusions** At high levels of PUFA, especially long chain n-3 PUFA and when pro-oxidants are released by mincing, shelf life is reduced. High dietary levels of vitamin E do not necessarily protect against this effect. Good shelf life was matched by high n-3 PUFA concentrations in grass-fed beef.

**Acknowledgements** This work was funded by DEFRA, ABN Limited, IFOMA, Tesco Stores Limited, Pedigree Petfoods, Rare Breeds Survival Trust and Roche Products Limited.

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# Effects of red clover silage and supplementary vitamin E on the polyunsaturated fatty acid content of milk from Holstein-Friesian cows

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**Introduction** Red clover silage is an important component of many organic dairy systems. The high intake and milk production potential of red clover silage has been recognised for many years (e.g. Thomas *et al.*, 1985). Our earlier studies confirmed this potential and showed additional benefits with increased polyunsaturated fatty acid (PUFA) content of milk (Dewhurst *et al.*, 2002). The objective of this study was to investigate further the effect of red clover silage on milk PUFA and to examine whether supplementary vitamin E, which is needed to slow oxidative deterioration of milk with enhanced PUFA content, had an effect.

**Materials and methods** Eight mid-lactation multiparous Holstein-Friesian dairy cows (initial weight=597 kg) were randomly allocated to receive one of four dietary treatments in a 3-period changeover design experiment. The four diets were a factorial combination of 2 forages (grass silage (GS) or red clover silage (RCS)) and 2 concentrates (normal or supplemental (+) levels of vitamin E). Concentrates were based on wheat (0.30), palm kernel expeller (0.15), maize gluten feed (0.14), rapeseed meal (0.11), sunflower meal (0.09), molasses (0.05), linseed meal (0.05), groundnut meal (0.05), soyabean meal (0.02), vegetable fat (0.015) and minerals/vitamins (0.025) and contained either 25 or 250 IU vitamin E per kg (as DL- $\alpha$ -tocopheryl acetate). Concentrates were fed at 8 kg/day through out-of-parlour feeders and forages were offered ad libitum through roughage intake control feeders (Insentec B.V., Marknesse, The Netherlands). Feed intake, milk yield and milk composition (Milkoscan 605) values from the final week of each 4-week period were used for the statistical analysis. Additional milk samples were taken during the final week of each period, bulked by cow, and analysed for fatty acid content using a one-step method. Results were analysed using REML (Genstat 5; Lawes Agricultural Trust, 2000) with a fixed model of 'crop'  $\times$  'vitamin E level' and a random model of 'period' + 'cow'.

**Results** The standard concentrate had the following composition: starch: 231 g/kg DM; neutral detergent fibre (NDF): 246 g/kg DM; crude protein (CP): 250 g/kg DM). Chemical analysis of GS and RCS gave the following values: for oven-DM: 335 and 220 g/kg; for CP: 199 and 202 g/kg DM; for NDF: 563 and 505 g/kg DM; and for oil: 43.2 and 46.2 g/kg DM respectively. The Table shows the effects of treatments on DM intake, milk production and milk composition.

**Table** Effects of forage crop and supplementary vitamin E on silage intake, milk production and milk composition.

	Dietary treatment <sup>(†)</sup> :				s.e.d. (crop)	Significance:	
	GS	GS+	RCS	RCS+		crop	vitamin E
Silage DM intake (kg/day)	9.21	9.50	12.68	12.81	0.329	***	NS
Milk yield (kg/day)	23.1	23.7	24.9	25.1	0.783	*	NS
Milk fat content (g/kg)	36.8	37.3	37.8	34.7	1.79	NS	NS
Milk protein content (g/kg)	29.0	28.7	29.4	29.9	0.12	***	NS
<u>Milk fatty acids (g/100g total fatty acids)</u>							
Linoleic acid (C18:2)	1.24	1.12	1.54	1.51	0.034	***	NS
$\alpha$ -linolenic acid (C18:3)	0.471	0.453	0.917	0.939	0.0193	***	NS
Vaccenic acid (C18:1, <i>trans</i> -11)	1.31	1.28	1.16	1.16	0.044	**	NS
Conjugated linoleic acid ( <i>cis</i> -9, <i>trans</i> -11)	0.447	0.475	0.404	0.383	0.0142	***	NS

<sup>(†)</sup> GS=grass silage; RCS= red clover silage; + = supplementary vitamin E in the concentrates (250 IU/kg).

There was no significant effect of forage crop on the concentrations (g/100g total fatty acids) of C14:0 (mean=10.38), C16:0 (mean=30.7), C18:0 (mean=12.16) or total C18:1 (mean=25.0) fatty acids.

**Conclusions** This study confirmed the higher intake characteristics and milk yields of RCS in comparison with GS and also showed a small increase in milk protein content. The study also confirmed the proportionately large increases in concentrations of C18:2 and C18:3 in milk from cows fed RCS. This and the significant reduction in concentrations of intermediates of rumen biohydrogenation (conjugated linoleic acid (*cis*-9, *trans*-11) and *trans*-vaccenic acid (C18:1, *trans*-11)) suggests that there may have been reduced rumen biohydrogenation when feeding RCS. Vitamin E had no effect on silage intake, milk production or milk PUFA.

**Acknowledgements** The financial support of DEFRA, the Milk Development Council, the European Union and the Government of Libya (studentship for RAM) is gratefully acknowledged.

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## Effect of breed and diet on total lipid and selected shelf-life parameters in beef muscle

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**Introduction** Some studies with beef cattle have shown that breed and diet affect tissue fatty acid composition and meat quality (Choi et al., 2000; Scollan et al., 2001). However, the effects of breed are often confounded with differences in growth rate and body composition. Diet also affects fatty acid composition, however, feed composition studies are often confounded by the use of mixed diets and few have compared all-forage with all-concentrate diets. This study, therefore, was designed to compare Aberdeen Angus and Holstein-Friesian breeds growing at similar rates and fed either all-forage or a high concentrate diet.

**Materials and methods** Sixteen Aberdeen Angus (AA) and 16 Holstein-Friesian (HF) steers (initial age and live weight 6 months and ~200kg, respectively) were allocated to one of two dietary treatments (*ad libitum* grass silage plus sugarbeet pulp shreds at *circa* 15% of the total dry matter (DM) intake or a barley-based, full-fat soya concentrate and chopped barley straw at a ratio of 70:30 on a DM basis), resulting in eight animals per breed per dietary treatment. Animals were weighed every 14 days and information used to regulate the intake of the concentrate animals in order to maintain similar growth rates between diets within breed. Animals were slaughtered at 14 months of age. Lipid oxidation and colour (L\*a\*b\*) shelf-life were measured after 7d of simulated retail display in modified atmosphere (MAP) (4°C, 700 lux for 18 h out of 24 h). Fatty acids (GC) and vitamin E (HPLC) were analysed from samples of *L. dorsi* removed 48h post-mortem. The data were analysed using a general analysis of variance using breed and diet as the main factors.

**Results** Cold carcass weights, conformation and fat class were affected by breed, AA were heavier, fatter and had better conformation. Silage-fed animals produced fatter carcasses compared with their concentrate-fed counterparts. Breed had little effect on muscle fatty acids, except DHA. Feeding silage resulted in a 50% increase in total fatty acids as well as higher levels of C18:3n-3, EPA, DPA and DHA. It also resulted in a low n-6:n-3 ratio but at the expense of the polyunsaturated to saturated fatty acid (P:S) ratio. HF had a higher P:S ratio compared with AA. In meat from concentrate-fed animals lipid oxidation was increased and by 7d colour was below acceptable. Vitamin E concentration in meat from silage-fed animals was more than twice that in meat from concentrate-fed animals.

	Breed		s.e.d.	P	Diet		s.e.d.	P
	AA	HF			Conc	Silage		
Carcass weight (kg)	99.3	90.3	2.60	**	94.3	95.3	1.71	NS
Conformation (1-155)	68.6	38.1	7.71	***	49.3	57.4	5.05	NS
Fat class (1-145)	55	30	4.1	***	32.2	53.2	4.06	***
Fatty acids (mg/100g muscle)								
Total fatty acids	2194	2111	139.3	NS	1724	2581	139.3	***
18:2 n-6	100.6	108.3	6.68	NS	146.9	62.0	6.68	***
18:3 n-3	18.3	20.9	1.60	NS	7.2	32.0	1.60	***
20:4 n-6	42.6	44.2	2.79	NS	53.6	33.1	2.79	***
20:5 n-3 (EPA)	11.0	11.2	1.05	NS	4.5	17.7	1.05	***
22:5 n-3 (DPA)	17.3	18.0	1.28	NS	10.8	24.5	1.28	***
22:6 n-3 (DHA)	2.7	3.6	0.30	**	1.3	5.0	0.30	***
P:S	0.15	0.18	0.010	**	0.24	0.09	0.010	***
n-6:n-3	4.9	5.3	0.24	NS	8.9	1.2	0.24	***
Lipid oxidation <sup>1</sup> (d7)	1.5	1.4	0.33	NS	2.4	0.5	0.15	***
Colour saturation (d7)	17.1	17.3	0.88	NS	16.2	18.3	0.57	**
Vitamin E <sup>2</sup>	2.3	2.2	0.39	NS	1.4	3.2	0.22	***

NS, not significant; \*\*\*P<0.001; \*\*P<0.01; <sup>1</sup>mg malonaldehyde/kg meat; <sup>2</sup>mg/kg meat

**Conclusion** Feeding grass silage rich in C18:3n-3 increased levels of this FA in beef muscle and increased the beneficial long-chain n-3 PUFA EPA, DPA and DHA. Despite the greater oxidative susceptibility of these longer-chain PUFA, the muscle Vitamin E concentration was sufficient to stabilise the meat. The faster maturing AA produced heavier, fatter carcasses with better conformation. HF muscle contained more PUFA resulting in a higher P:S ratio compared with muscle from AA.

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## Changes in carcass composition with growth and the effect of feed type in lambs of two breeds and their cross

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**Introduction** To enable producers to meet market requirements for lamb carcasses from the resources available, it is important to know how breeds differ in growth and development over time and how this is affected by feeding regime. The aims of this study were to explore the effect of diverse breed and feed types on carcass composition, growth and feed intake and investigate how these change during growth.

**Methods** Lambs of three breed types [24 Scottish Blackface (B), 28 Suffolk (S), and 33 reciprocal crosses between these breeds (X)] were grown to a target weaning weight (proportionally, 0.20 of estimated mature weight) or 8 weeks of age, whichever came sooner. At weaning, lambs were assigned randomly to *ad libitum* feeding of either a high quality performance test ration (PTR), designed not to limit growth, or a bulky feed. Feed intakes and live weights were recorded weekly thereafter. On reaching 0.30, 0.45 and 0.65 (end of test) of their mature weight, lambs were scanned using X-ray computed tomography (CT) from which their carcass fat, muscle and bone weights and contents were estimated. The derived variables evaluated were average daily gain in live weight and in individual tissues (ADG; g/day), average daily intake (ADI; g/day), and feed efficiency [ $1000 \times (\text{ADG}/\text{ADI})$ ]; these values were calculated between proportions of mature weight (start to 0.30, 0.30 to 0.45 and 0.45 to 0.65). Based on preliminary analysis, a general linear model (GLM) including breed-type, feed and sex as fixed effects, and their two-way interactions, was selected and fitted (Genstat 5, 2001) to describe the derived variables. Birth weight was included as a covariate in the model fitted.

**Results** As shown in Table 1, breed-type did not significantly affect fat proportion at any stage of maturity. At 0.45 mature B lambs had higher bone proportions ( $P < 0.05$ ) and at 0.65 mature had lower muscle proportions ( $P < 0.01$ ) than the other breed-types. Lambs fed the bulky feed had less fat and more muscle than those fed PTR ( $P < 0.001$ ). At 0.45 mature, compared to the other breed-types, S lambs showed a smaller difference in fat proportion between lambs fed the two feeds ( $P < 0.05$ ). ADG in fat, muscle and bone weights were lower for B lambs than S ( $P < 0.05$ ). X lambs had tissue ADGs closer to S than B. PTR fed lambs gained fat weight faster than those on bulky feed ( $P < 0.001$ ) and, between 0.30 and 0.45 mature weight, PTR fed lambs also gained muscle weight faster than those on bulky feed ( $P < 0.01$ ). B lambs had lower ADG in live weight than S and X lambs ( $P < 0.01$ ), and all three breed-types differed in ADI ( $P < 0.001$ ). B lambs had the lowest and S lambs the highest ADI, with X lambs having intermediate intakes. Lambs on the bulky feed had lower ADG, higher ADI and lower efficiency than lambs on PTR at all stages of maturity ( $P < 0.05$ ), however these differences became smaller as lambs grew towards maturity. A breed-type-feed interaction existed for ADI where B lambs had smaller differences in ADI between the two feeds than the other breed-types ( $P < 0.01$ ). Even in the presence of interactions, there were consistent differences in the derived variables between breed-types across feeds and between feeds across breed-types.

Table 1 Least square means of fat, muscle and bone as proportions of total tissue weight (g/kg) at each scanning event for different breed and feed types (within effect, means with different superscripts are significantly different ( $P < 0.05$ )).

Effect		0.30 mature			0.45 mature			0.65 mature		
		Fat	Muscle	Bone	Fat	Muscle	Bone	Fat	Muscle	Bone
Breed	B	152	621	227	262	557	181 <sup>a</sup>	375	475 <sup>a</sup>	150
	X	150	622	228	265	559	176 <sup>b</sup>	366	484 <sup>a</sup>	150
	S	142	628	230	260	566	174 <sup>b</sup>	357	498 <sup>b</sup>	145
Feed	PTR	178 <sup>a</sup>	610 <sup>a</sup>	212 <sup>a</sup>	288 <sup>a</sup>	545 <sup>a</sup>	167 <sup>a</sup>	392 <sup>a</sup>	465 <sup>a</sup>	143 <sup>a</sup>
	Bulky	118 <sup>b</sup>	638 <sup>b</sup>	244 <sup>b</sup>	237 <sup>b</sup>	576 <sup>b</sup>	187 <sup>b</sup>	339 <sup>b</sup>	507 <sup>b</sup>	154 <sup>b</sup>
	Max s.e.d.	9.53	7.15	5.7	7.14	6.51	2.91	7.66	6.35	2.36

**Conclusions** Little significant breed differences in carcass composition were observed although it appeared that B had higher bone and lower muscle proportions than S later in growth. Fat proportion in S lambs was less sensitive to feed nutritional value in comparison to B or X lambs. S lambs had faster growth in live weight and individual tissues than B lambs. The bulky feed generally lead to lambs being less fat, with lower rates of fat and muscle accretion, and slower daily gain in live weight than a feed designed to be non-limiting to growth. In addition, although lambs fed on bulky feed had higher feed intakes and lower efficiencies, the constraint on performance of lambs on bulky feed lessened as lambs matured.

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**Acknowledgements** Thanks to SEERAD for funding, The Worshipful Company of Woolmen postgraduate award to J.M. Macfarlane and SAC staff for their technical input to the study.

## Modeling growth of lambs of two breeds and their cross on different feed types

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**Introduction** In order for producers to meet market requirements for lamb carcasses from the resources available, they need to know how breeds differ in growth and development over time and how this is affected by feeding regime. A previous paper explored the effect of diverse breed and feed types on carcass composition, growth and feed intake. This paper uses different mathematical descriptions of growth to study the relationships between live weight and time and between live weight and cumulative feed intake.

**Methods** Lambs of three breed-types [24 Scottish Blackface (B), 28 Suffolk (S) and 33 reciprocal crosses between these breeds (X)] were weighed weekly from birth and grown to a target weaning weight (proportionally, 0.20 of estimated mature weight) or 8 weeks of age, whichever came sooner. At weaning, lambs of each breed were assigned randomly to *ad libitum* feeding of either a high quality performance test ration (PTR), designed not to limit growth, or a bulky feed. Feed intakes were recorded weekly thereafter. A Gompertz function was fitted to average live weight data for each breed-type-sex group of lambs on PTR using nonlinear regression to describe growth over time (Genstat 5, 2001). The form of the function fitted was  $W=A(\exp(-\exp(G_0-Bt)))$  where  $W$  is live weight at time  $t$ ,  $A$  is mature weight,  $G_0$  is an initial condition and  $B$  is a rate parameter. Due to the high correlation between estimates of  $A$  and  $B$ , as in Lewis *et al.* (2002), the lumped parameter  $Z=A*B$  was obtained along with  $Z/e$ , an estimate of maximum daily gain. The Spillman function (see Parks, 1982) was fitted to average data for each breed-type-feed-sex group using nonlinear regression to describe growth as a function of cumulative feed intake ( $F$ ) as  $W=W_0+(A-W_0)(1-\exp(-kF))$  with  $W_0$  as starting weight. Again, due to high correlation among estimates, a lumped parameter ( $Ak$ ) was derived, which approximates a measure of efficiency.

Table 1 Maximum daily gain ( $Z/e$ ; g/day) and  $Z$  (kg/day) estimated by the Gompertz function and  $Ak$ (g/kg) estimated by the Spillman function for lambs of each breed type-feed-sex group

Breed	Feed	Sex	$Z/e$	$Z$	$Ak$
B	PTR	M	314	0.8546	0.4089
		F	255	0.6933	0.3916
	Bulky	M	-	-	0.2236
		F	-	-	0.2117
X	PTR	M	393	1.0692	0.3849
		F	324	0.8803	0.3860
	Bulky	M	-	-	0.2224
		F	-	-	0.2267
S	PTR	M	443	1.2029	0.4196
		F	349	0.9498	0.3884
	Bulky	M	-	-	0.2135
		F	-	-	0.2147

Neither S.E.s nor the significance of differences are shown as they are misleading due to high correlations between the parameter estimates.

**Results** The Gompertz function fitted the data well (r.s.d. 0.443 to 0.790 kg) for all groups. B lambs had lower  $Z$  and  $Z/e$  values than S lambs. X lambs had values closer to those of S than of B lambs (Table 1). Female lambs had lower  $Z$  and  $Z/e$  values than male lambs. The Spillman function also fitted the data well for all groups with r.s.d. lower than those of the Gompertz function (0.219 to 0.558 kg). Breed and sex did not have important effects on  $Ak$ . Lambs on bulky feeds had lower  $Ak$  values than lambs on PTR.

**Conclusions** S lambs had faster growth than B lambs. X lambs were more similar to S lambs than B lambs in their characteristics of growth as a function of time. Lambs fed on bulky feed were less efficient than lambs on PTR. Although differences in growth did exist between lambs of different breeds and sexes, this did not lead to differences in efficiency. Both the Gompertz

and Spillman functions model growth well. The Spillman function can be useful where growth is constrained by the food on offer, and thus not appropriately described by a function of the Gompertz form.

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**Acknowledgements** Thanks to SEERAD for funding, The Worshipful Company of Woolmen postgraduate award to J.M. Macfarlane and SAC staff for their technical input to the study.

# Predicting genetic gain when rates of inbreeding are constrained to pre-defined values

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**Introduction** Dynamic selection algorithms using quadratic indices to optimise the contributions of selection candidates for maximising rates of genetic gain ( $\Delta G$ ) while constraining the rate of inbreeding ( $\Delta F$ ) in the long-term to pre-defined values, are available (Grundy *et al.*, 1998). Avendaño *et al.* (2001 *a,b*) applied these optimal selection algorithms on the UK Meatlinec (sheep) and Aberdeen Angus (beef cattle) pedigree breeds and found substantial expected increases (of at least 17%) in the average index score at the observed  $\Delta F$ . Although these algorithms constitute powerful operational tools for breeding schemes, the framework for deterministically predicting  $\Delta G$  under optimal selection with restricted  $\Delta F$  is not yet available. This study presents a novel approach to this problem.

**Methods** The theoretical ideal solution for maximising  $\Delta G$  with constrained  $\Delta F$  could be achieved by an exact linear allocation of the long-term genetic contributions ( $r$ ) of selected candidates according with their Mendelian sampling terms ( $a$ ) (Grundy *et al.*, 1998). Under such optimal solution an idealised rate of gain ( $\Delta G_{ideal}$ ) can be predicted in terms of the number of candidates per generation ( $T$ ), the heritability ( $h^2$ ) and  $\Delta F$  as  $\Delta G_{ideal} = i(k)^{-1}(\frac{1}{2} h^2)^{\frac{1}{2}}$ , where  $i$  is the selection intensity and  $k$  is the variance reduction parameter for a selection proportion ( $p$ ) satisfying  $(8T\Delta F)^{-1} = 2p(i-x)^2(1+x^2-ix)^{-1}$ . The ideal outcome is not attainable since  $a$  are not known and  $r$  evolves after selection. An approximate expression can be obtained ( $\Delta G_{ub}$ ) as  $\Delta G_{ub} = \rho \Delta G_{ideal}$ , where  $\rho$  is the accuracy of the Mendelian sampling term for selected individuals (Grundy *et al.*, 1998). Accurate predictions of  $\rho$  were obtained by extending the Pseudo-BLUP index of Wray and Hill (1989) to allow three new sources of information; the maternal and paternal estimated  $a$ , and the average estimated  $a$  of the dams mated to the sire. Phenotypic and genetic co-variance matrices were derived for predicting  $\rho$  at convergence of long-term genetic contributions of selected candidates. The  $\Delta G_{ub}$  was compared with empirical rates of gain ( $\Delta G_{sim}$ ) obtained from stochastic simulations of schemes under optimised BLUP selection. Discrete generation populations were simulated for a range of  $h^2$  and two inbreeding constraints ( $\Delta F=0.01$  or  $0.025$ ).

**Results** For a population size of 100 candidates per generation, the  $\Delta G_{ub}$  calculated after predicting  $\Delta G_{ideal}$  and  $\rho$ , clearly provided an accurate prediction of  $\Delta G_{sim}$  (Figure 1). This was true for both levels of  $\Delta F$  and for almost the whole range of  $h^2$ . For a population size of 300, the  $\Delta G_{ub}$  predicted  $\Delta G_{sim}$  well, although  $\Delta G_{sim}$  was more sensitive to changes in  $\Delta F$  than  $\Delta G_{ub}$  which was underestimated by about 10% for  $\Delta F=0.01$ , and overestimated by about 13% for  $\Delta F=0.025$  (Figure 2). The only inputs required for  $\Delta G_{ub}$  were the amount of resources ( $T$ ), the target  $\Delta F$  and  $h^2$ .

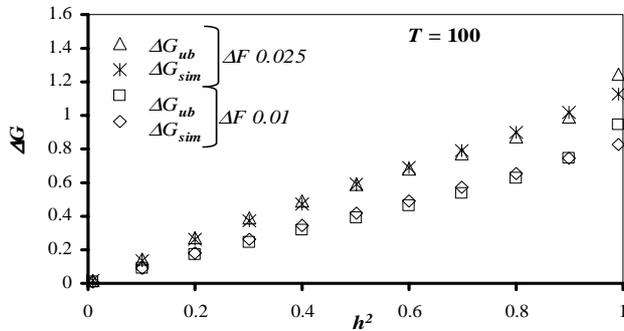


Figure 1. Comparison of  $\Delta G_{ub}$  and  $\Delta G_{sim}$  for  $T=100$

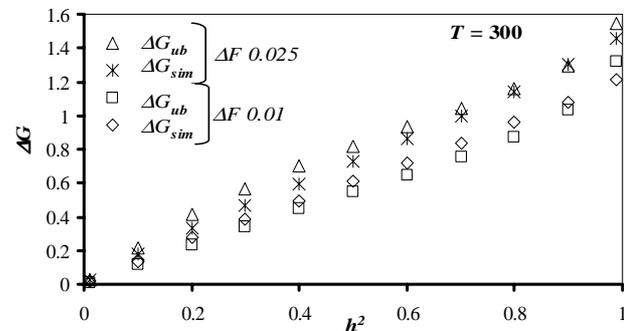


Figure 2. Comparison of  $\Delta G_{ub}$  and  $\Delta G_{sim}$  for  $T=300$

**Conclusions** This is the first completely deterministic prediction for  $\Delta G$  in schemes under BLUP optimal selection with target  $\Delta F$ . These predictions are relevant for the optimum design of breeding programs, providing the necessary accompanying tool for the available operational dynamic optimisation algorithms.

**Acknowledgments** The Meat Livestock Commission (MLC), DEFRA and BBSRC are greatly acknowledged.

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## Developing a UK Dairy Fertility Index

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**Introduction** National dairy records have shown that correlations between production and fertility are generally unfavourable. There has therefore been a genetic downward trend for fertility due to the increase selection pressure on yield and as a genetic problem it requires a genetic solution. The effect of sire genetics on daughter fertility is included in many genetic indices around the world. As part of a DEFRA LINK project to produce an UK fertility index this study estimates genetic parameters and sire predicted transmitting ability (PTAs) for fertility traits in the UK.

**Materials and Methods** Insemination, calving, body condition score (CS) and milk records were extracted from National Milk Records (NMR) and Holstein UK (HUK) databases for cows commencing first lactation from 1992 to 2000. A number of fertility traits, and associated traits, were produced from these records, namely CI (days), days in milk until first insemination (DIM1, days), non-return rate after 56 days (NR56, 0/1), number of inseminations per calving (CINS, count), milk yield at day 110 (MILK, kg) and CS (0-9, adjusted). Limits were used on the data to screen out recording errors (see Wall *et al*, 2002) and insemination records validated. More stringent limits were placed on data for genetic parameter estimation (28,747 records for 986 sires) than for sire PTA estimation (approx. 1.4 million records for 18,000 sires). Variance components were estimated jointly by REML with a sire-maternal grandsire model using VCE4 (Groneveld, 1998) and are shown in Table 1. The genetic parameters above were used in a multi-trait best linear unbiased prediction (BLUP) analysis using PEST (Groneveld *et al*, 1990) to obtain sire PTAs for each trait.

**Table 1.** Heritability (diagonal), genetic (above diagonal) and phenotypic (below diagonal) correlations, mean, standard deviation (s.d.), sire variance ( $\sigma_s^2$ ) and sire PTA range (bad→good) for CI, CS, MILK, DIM1, NR56 and CINS.

	CI	CS	MILK	DIM1	NR56	CINS	Mean	s.d.	$\sigma_s^2$	PTA Range
CI	<b>0.047</b>	-0.334	0.287	0.762	-0.363	0.626	387.7	50.2	28.13	17.88→-10.28
CS	-0.052	<b>0.240</b>	-0.394	-0.562	-0.077 <sup>#</sup>	0.023 <sup>#</sup>	4.4	1.7	0.12	1.27→-0.88
MILK	0.053	-0.149	<b>0.278</b>	0.500	-0.153 <sup>#</sup>	0.031 <sup>#</sup>	23.9	5.4	1.18	-4.5→3.67
DIM1	0.481	-0.091	0.043	<b>0.048</b>	0.214 <sup>#</sup>	-0.004 <sup>#</sup>	81.4	29.6	8.55	10.05→-7.83
NR56	-0.346	0.014	-0.031	0.021	<b>0.018</b>	-0.899	1.7	0.5	0.001	-0.17→0.13
CINS	0.674	-0.001	0.045	-0.064	-0.677	<b>0.028</b>	1.7	1.0	0.007	0.13→-0.14

<sup>#</sup>Estimates not statistically different from zero

**Results** The genetic parameters (Table 1) are consistent with those reported previously in the UK and internationally. There is a negative genetic correlation between CS and MILK with CI and DIM1, indicating cows in poor condition and /or high yielding are genetically more likely to have a long DIM1 and subsequently a longer CI. There is a strong genetic correlation between NR56, CINS and CI indicating that improving one trait will lead to a correlated improvement in the others. Table 1 shows a wide genetic variation (PTA) in the fertility traits thus presenting an opportunity to select on and improve fertility traits. The correlation between sire CI PTA and the current UK dairy profit index, £PLI, is 0.3 indicating that it is more likely to select bulls with an unfavourable CI PTA (positive). However this relationship is not 1 and Figure 1 shows that there is a range of bulls with a high £PLI that also have favourable CI PTAs. The correlation between DIM1 and £PLI is 0.48 and the correlation between NR56 and CINS with £PLI is low and unfavourable.

**Conclusion** Improving fertility genetically is an important objective for dairy farmers for long term sustainability, profitability and welfare of the cow. This study shows that genetic parameters and PTAs can be calculated using data that are currently available. These results show that there is a wide scope for including fertility in the selection of bulls. There are a number of high £PLI bulls that also have poor fertility PTAs but some high £PLI bulls have average to good fertility PTAs. The bulls with poorer fertility PTAs could be removed from the selection process and a wide selection of good “production” bulls with reasonable fertility would remain to select from and thus help to slow down or even halt the continued decline in fertility traits.

**Acknowledgements** We thank DEFRA, NMR, HUK, Dartington Trust, CIS, Cogent and Genus for their support

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## The use of faecal inocula for estimating the *in vitro* digestibility of horse feeds

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**Introduction** There is a dearth of information available on the effects of donor animal on the fermentative capacity of equine faecal inocula for use in *in vitro* digestibility determinations. Furthermore, there is little knowledge of the degradation characteristic of feedstuffs incubated with equine faecal inocula. As such this study aimed to elucidate the effect of donor animal on the fermentation of feedstuffs *in vitro* and to assess the *in vitro* degradation characteristics of three commonly fed components of horse diets incubated with equine faecal inocula.

**Materials and Methods** Seven mature Welsh-cross pony geldings provided the faecal inocula in a cross-over design experiment whereby four ponies (group 1) were fed a low-starch diet (diet A) and three (group 2) were fed a high-starch diet (Diet B) in a 50:50 ratio with mature grass hay to give a total daily DM intake of 1.75% of LW/d. Each diet was fed in two equal meals for 14 days, after which group 1 were offered diet B and group 2 offered diet A. At the end of each 14 day period, freshly voided faeces were collected from each animal and used as inocula for *in vitro* digestibility determinations, using the gas production technique of Theodorou *et al.* (1994), to assess the ability of each inoculum to ferment the mature grass hay, the low-starch mix (LS) or the high-starch mix (HS) *in vitro*. Gas production was measured over a 54 h period, after which *in vitro* dry matter loss (DML) was determined by lyophilization of the fermentation residues. Gas production curves were fitted to the model of France *et al.* (1993) to determine the total gas volume (A), lag time ( $L_T$ ), fractional rate of degradation (FRGP) and the time taken to produce 50 % of the total gas production ( $T_{50}$ ). Data were analysed by REML as a cross-over/split-plot design using Genstat 5 (2000).

**Results** The results of this study showed no effect of donor animal on the fermentative capacity of the faecal inocula. Mathematical analysis of the gas curves (table 1) showed no effect of donor animal on the rate or extent to which the substrates were degraded. However, the substrates themselves, H, LS and HS, showed significant differences in the extent and rate at which they were fermented ( $P < 0.001$ ) (table 2).

**Table 1:** Mean gas production parameters; total gas volume (A), lag time ( $L_T$ ), fractional rate of degradation (FRGP), time taken to produce 50 % of A ( $T_{50}$ ) and dry matter loss (DML) for the faecal inocula generated from each pony

	Pony 1	Pony 2	Pony 3	Pony 4	Pony 5	Pony 6	Pony 7	s.e.d.	Sig.
A (ml)	186.3	199.0	181.8	187.4	185.4	184.4	192.6	11.24	ns
$L_T$ (h)	1.362	1.757	1.172	1.458	1.278	1.308	1.181	0.3527	ns
FRGP ( $h^{-1}$ )	0.0947	0.0985	0.0922	0.0866	0.0930	0.0920	0.0807	0.01309	ns
$T_{50}$ (h)	10.95	10.48	11.33	12.02	10.93	11.40	13.30	1.305	ns
DML (%)	57.45	61.38	57.78	60.38	60.77	59.18	57.40	1.827	ns

**Table 2:** Mean gas production parameters and DML for grass hay (H), low-starch mix (LS) & high-starch mix (HS) incubated with equine faecal inocula

	H	LS	HS	s.e.d.	Sig.
A (ml)	143.5 <sup>a</sup>	151.4 <sup>a</sup>	269.4 <sup>b</sup>	7.414	$P < 0.001$
$L_T$ (h)	0.709 <sup>a</sup>	1.376 <sup>b</sup>	1.993 <sup>c</sup>	0.1760	$P < 0.001$
FRGP ( $h^{-1}$ )	0.0527 <sup>a</sup>	0.0952 <sup>b</sup>	0.1254 <sup>c</sup>	0.00514	$P < 0.001$
$T_{50}$ (h)	14.09 <sup>a</sup>	10.40 <sup>b</sup>	9.98 <sup>b</sup>	0.4593	$P < 0.001$
DML (%)	48.47 <sup>a</sup>	49.67 <sup>a</sup>	79.43 <sup>b</sup>	1.072	$P < 0.001$

**Conclusion** In this study donor animal had little effect on the *in vitro* digestibility determinations of the feedstuffs investigated. However, the feedstuffs themselves were remarkably different in their fermentation characteristics and the gas production technique appeared to be a valuable tool for evaluating the *in vitro* degradation characteristic of these feedstuffs for horses.

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

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# An *in vitro* assessment of amino acid requirements for optimal xylan fermentation by mixed ruminal micro-organisms from the sheep rumen

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**Introduction** Ruminal microbes play a important role in the fermentation of structural plant polysaccharides, and constitute a major source of protein for the animal. Dietary protein provides amino acids which generally stimulate microbial growth rates and yields. The aim of this experiment was to identify, using a deletion approach, which individual amino acids limit fermentation of one of the main components of plant fibre, xylan.

**Materials and methods** Incubations of ruminal fluid were carried out as described by Atasoglu *et al.* (2002). Ruminal fluid was taken, after overnight starvation, from four ruminally cannulated sheep receiving a mixed grass hay/concentrate diet. Equal volumes were mixed and diluted 3-fold with a minerals/bicarbonate buffer. NH<sub>4</sub>Cl was added to all incubations to give a final concentration of 10 mmol/l. Amino acids, a mixture of 20 amino acids, or mixtures with single amino acid deletions, were added to a final concentration of 0.25 g/L of each amino acid. 30 ml of the mixtures were added to 100-ml glass syringes containing 200 mg of oat spelts xylan (Sigma). Two syringes were set up for each treatment, with or without added energy source. Gas production was measured at 2, 4, 6, 8, 12, 18 and 24 h. Initial and final samples were collected and analysed for NH<sub>3</sub>, VFA, sugar and total cell N. Data were analysed by ANOVA with the repeat incubations and the

**Table 1** Influence of single amino acid deletions on the fermentation of xylan by ruminal micro-organisms *in vitro*

	Gas produced (ml)		Total VFA (mM)
	12	24	24
Incubation time (h)...			
Amino acid supplementation	Mean	Mean	Mean
No amino acids (no AA)	25.6	41.0	40.62
All amino acids (AA)	30.2	41.8	66.57
Amino acid deletion from			
Glutamate	30.3 <sup>a</sup>	40.5	61.18 <sup>b</sup>
Tyrosine	26.7 <sup>b</sup>	39.2 <sup>b</sup>	66.35
Alanine	30.3 <sup>a</sup>	41.3	63.04
Serine	29.5 <sup>a</sup>	40.8	67.40
Glutamine	30.5 <sup>a</sup>	41.7	63.52
Valine	29.5 <sup>a</sup>	40.8	65.34
Methionine	28.5 <sup>a</sup>	40.3	65.90
Histidine	29.7 <sup>a</sup>	40.3	65.19
Cysteine	29.3 <sup>a</sup>	40.4	70.19
Lysine	30.0 <sup>a</sup>	41.8	66.39
Tryptophan	27.6 <sup>a,b</sup>	38.2 <sup>a,b</sup>	65.27
Glycine	29.7 <sup>a</sup>	41.8	65.05
Phenylalanine	28.1 <sup>a,b</sup>	39.8	65.31
Threonine	28.8 <sup>a</sup>	39.8	64.74
Asparagine	30.2 <sup>a</sup>	41.2	65.62
Proline	30.1 <sup>a</sup>	40.8	64.12
Leucine	27.8 <sup>a,b</sup>	39.5	66.27
Isoleucine	30.4 <sup>a</sup>	40.9	64.05
Aspartate	30.0 <sup>a</sup>	40.5	64.44
Arginine	28.5 <sup>a</sup>	39.2 <sup>b</sup>	65.24
<b>SEM</b>	0.08	0.04	1.39

Mean values calculated from six incubations. Values with superscript 'a' or 'b' in the same column are significantly different ( $P < 0.05$ ) from no AA and AA treatments respectively

amino acid deletion/addition as treatment effects, using Genstat 6<sup>th</sup> edition (Lawes Educational Trust, Rothamsted, Herts, UK). Treatment means were then compared by a paired t-test.

**Results** The addition of 20 amino acids to the fermentation increased gas production at 12 h by 0.18 and increased VFA concentration by 0.64 ( $P < 0.005$ ). The individual deletion of 5 amino acids significantly ( $P < 0.005$ ) decreased gas production at 12 h and 24 h by 0.08 to 0.12 of the rate observed with all 20 amino acids (Table 1). The effects were greatest for tyrosine, tryptophan and leucine. Glutamic acid was the only amino acid whose omission caused a significant ( $P < 0.005$ ) decrease in total VFA production. There was significantly ( $P < 0.005$ ) higher production of NH<sub>3</sub> in treatments where amino acids were added. Microbial growth yield was significantly ( $P < 0.05$ ) improved by addition of 20 amino acids, from 0.019 to 0.040 g N formed/g sugar utilised. However, growth yields with single amino acid deletions were not significantly different from those obtained in the presence of all 20 amino acids, although the deletion of glycine approached significance ( $P = 0.069$ ).

**Conclusion** There appears to be no single amino acid that primarily limits microbial growth rate or yield during fermentation of xylan. However, the aromatic amino acids, notably tyrosine and tryptophan, as well as leucine appear to be most-limiting for microbial growth.

**Acknowledgements** We thank the Commonwealth Scholarship Commission in the UK for the award of a scholarship to AYG, the Ministry of Education, Republic of Turkey for a scholarship awarded to CA. The Rowett Research Institute receives funding from SEERAD.

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# Predicting maize silage starch degradability by near infrared reflectance spectroscopy

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**Introduction** Maize silage consists of a starch and a fibrous fraction, both of which should be considered when assessing nutritive value. The *in vitro* evaluation of starch disappearance is laborious and costly. The near infrared reflectance spectroscopy (NIRS) technique requires limited sample preparation and is quick to operate once a calibration is established. This study investigated the potential of NIRS to predict maize starch disappearance *in vitro*.

**Materials and Methods** Thirty one maize silages, milled (1 mm) and dried (24h at 60°C), were evaluated for starch disappearance *in vitro* at 4, 8, 12 and 24 hours using a modified first stage microbial incubation (Tilley and Terry, 1963). Starch content of the whole sample and that of the undigested residues were determined using the GOD Perid Colorimetric Test Kit (Boehringer Cat. N°. 124036) following an incubation with amyloglucosidase. Starch disappearance values were fitted to the model of Ørskov and McDonald (1979). The NIRS calibrations were developed from six replicate scans (1100 to 2500 nm), following the removal of residual moisture (4h at 60°C), using a NIRSystems 5000 monochromator (Foss UK Ltd). The spectral data was stored as log 1/reflectance (R), meaned and corrected for particle size effect and spectral curvature using the standard normal variate and detrending (SNV-D) procedure. The SNV-D data was then derivatised using the 1,4,4,1 mathematical treatments. Calibrations were developed using the partial least squares (PLS) and modified partial least squares (MPLS) procedures.

**Results** Starch content varied from 81 to 346 g/kg dry matter (DM) reflecting differing plant maturities. MPLS generally provided higher calibration and validation statistics. Starch content of maize silage was accurately predicted ( $R^2$  0.957) and the accuracy of prediction of starch disappearance at specific time points increased with increasing incubation time. The best calibration was developed using the MPLS statistical model at 24h. The 'a' and 'b' fractions exhibited little variation between samples, and as such all predictive equations were poor. Only the fractional rate of disappearance and the lag phase demonstrated any variation. The MPLS procedure generated a reasonable calibration for the prediction of the 'c' fraction although the cross-validation  $R^2$  was weak (1-VR 0.492). Lag phase was poorly predicted.

**Table 1** NIRS calibration and cross-validation statistics (as g/kg starch unless otherwise stated).

Parameter	Range	PLS				MPLS			
		SEC	$R^2$	SECV	1-VR	SEC	$R^2$	SECV	1-VR
Starch (g/kg DM)	81-346	15	0.951	17	0.934	14	0.957	17	0.937
Disappearance data									
4h	146-409	36	0.268	38	0.197	36	0.262	38	0.185
8h	321-630	41	0.496	46	0.366	43	0.457	46	0.369
12h	516-753	36	0.646	45	0.461	28	0.793	39	0.597
24h	861-993	16	0.737	18	0.657	14	0.784	17	0.684
Disappearance kinetics									
a fraction	0-13	1.81	0.169	1.94	0.091	1.73	0.240	1.89	0.138
b fraction	987-1000	1.81	0.169	1.94	0.091	1.73	0.240	1.89	0.138
c fraction	0.068-0.122	0.007	0.595	0.008	0.469	0.005	0.725	0.007	0.492
Lag (h)	0.07-0.73	0.146	0.113	0.153	0.030	0.127	0.255	0.136	0.141

SEC = Standard error of calibration, SECV = Standard error of cross validation, 1-VR = coefficient of determination

**Conclusion** Despite the limited number of samples used within this study, NIRS has demonstrated the potential to accurately and rapidly predict the *in vitro* starch disappearance of maize silage at extended incubations (>12 h) and to generate reasonable SEC and SECV (0.0046 and 0.0065 respectively of the mean) values for the fractional rate of starch disappearance. Calibrations such as this offer the potential to incorporate the kinetics of substrate degradation into future mechanistic rationing systems and allow the development of concentrate rations designed to complement home-grown feedstuffs high in starch. Further work needs to be conducted on a larger population size, as well as investigating the effect of sample preparation on subsequent calibration statistics.

**Acknowledgements** This work was funded by an award from the Society of Feed Technologists and the author was a recipient of a MAFF studentship.

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# Estimation of microbial N yield on red clover silages supplemented with barley by rumen simulation technique (RUSITEC)

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**Introduction** Despite many promising characteristics as a low-input home-grown protein source, red clover (Cv) silage supplies deficient amounts of rumen available carbohydrates to allow an efficient use of the available forms of soluble N. An *in vitro* experiment was designed in order to determine the optimum rate of inclusion of a rumen degradable carbohydrate source to optimise rumen microbial N yield of Cv silage.

**Material and methods** Red clover silage compared to ryegrass (Ry) silage was used as the basal diet to assess the microbial N yield under increasing levels of ground barley grain inclusion (0, 15, 30 and 45 % on a dry matter (DM) basis). Treatments were replicated in three independent 10-day incubation periods (blocks). The study was carried out using RUSITEC (Czerkawski and Breckenridge, 1977) consisting of 8 vessels each with a volume of 1 l. Rumen liquor and digesta were collected from a rumen fistulated dairy cow fed with concentrate (8 kg/day) and free access to ryegrass silage. Artificial saliva was infused at a rate of 0.5 ml/min (i.e. 700 ml/day) and on day 4 of incubation,  $^{15}\text{NH}_4\text{SO}_4$  (0.56 g) was added as a microbial marker. The system was initiated and maintained as described by Czerkawski and Breckenridge (1977). Feed residues from day 5 until the end of the experiment were used to calculate DM disappearance by freeze drying. On day 8, every vessel was sampled (at 0, 1, 2, 3, 4, 6, 8, and 24 h) for analysis of pH, and  $\text{NH}_3\text{-N}$ . From day 9, effluent collection bottles were placed in a chilled water bath. On day 10, the dacron bags were removed and squeezed by hand without buffer, and the liquids were returned to the vessels. Solid associated bacteria (SAB) were extracted from the digesta residues left into the dacron bags by pummeling in a Stomacher (Seward, UK) twice with 150 ml saline solution (0.9 % NaCl) for 5 minutes. The total volume of the effluents collected (i.e. vessel and SAB extracts) was recorded, and proportionally subsampled to give a 500 ml composite sample. Composite and background samples (taken prior  $^{15}\text{N}$  labelling) were used to determine bacterial yield. Feed residues were removed by centrifuging at  $1500 \times g$  for 10 minutes ( $4^\circ\text{C}$ ); supernatants removed were centrifuged at  $30,000 \times g$  for 15 minutes ( $4^\circ\text{C}$ ), and the resultant pellet was washed twice in saline solution and washed twice in distilled water to remove salts and ammonia N. Pellets were resuspended in a minimum amount of water, freeze dried and weighed. To obtain the whole pellet weight and sample, the procedure was repeated as for the bacterial pellet, but avoiding the first centrifugation used to remove feed residues ( $1500 \times g$ , 10 min,  $4^\circ\text{C}$ ). Data were analysed as a factorial analysis of variance ( $2 \times 4$ ; two species and 4 levels of grain) as a complete block design with polynomial contrasts using Genstat, and significance was declared at  $P \leq 0.05$ .

**Results** The chemical composition of Ry (DM = 312 g/kg fresh matter (FM); crude protein (CP) = 139 g/kg DM; water soluble carbohydrates (WSC) = 71 g/kg DM;  $\text{NH}_3\text{-N}$  = 133 g/kg N; pH = 4.23) and Cv (DM = 296 g/kg FM; CP = 168 g/kg DM; WSC = 28 g/kg DM;  $\text{NH}_3\text{-N}$  = 80 g/kg N; pH = 4.12) silages were typical. Ammonia N and pH were above 50 mg/L and 6.6 respectively in all treatments and at all times monitored. No differences in DM (DMD) or organic matter (OMD) digestibility were detected between either silage (Table 1), but neutral detergent fibre (NDF) digestibility of Cv silage was significantly lower than for Ry. Inclusion of barley grain linearly increased DMD, OMD and bacterial yield. However, there was no clear response in N bacterial yield from Ry silage supplemented with barley, but in Cv silage, N bacterial yield increased up to 30 % barley inclusion.

**Table 1.** Dry matter, organic matter, NDF, nitrogen disappearance and bacterial and N bacterial yield from ryegrass silage and red clover silages supplemented with increasing proportions of barley grain in 48 hours *in vitro* incubations.

Species:	Ryegrass silage				Red clover silage				SEM <sup>1</sup>	P <sup>2</sup>
	0	15	30	45	0	15	30	45		
Percentage of grain:	0	15	30	45	0	15	30	45		
Dry matter digestibility (g/kg DM)	785	813	803	839	795	801	826	842	9.8	Gr lin
Organic matter digestibility (g/kg DM)	782	811	798	835	789	799	823	843	10.6	Gr lin
NDF digestibility (g/kg DM)	683	694	634	669	596	582	601	572	18.8	Sp
Bacterial yield (g/kg OMD)	285	285	308	359	324	328	376	371	29.1	Gr lin
N bacterial yield (g/kg OMD)	18.3	18.3	16.8	19.8	19.3	22.3	26.0	25.3	0.99	‡

<sup>1</sup> Standard error of the mean, n = 3.

<sup>2</sup> Significant factors,  $P < 0.05$ ; Gr, grain; Sp, species (ryegrass or red clover silage); lin, linear; quad, quadratic; ‡ Sp, Gr, Gr lin, Sp  $\times$  Gr, Sp  $\times$  Gr lin, Sp  $\times$  Gr quad.

**Conclusions** It is concluded that 30 % inclusion of barley grain in Cv silage maximises rumen N bacterial yield, and that in Ry silage no response was observed to barley inclusion.

**Acknowledgements** This work was funded by DEFRA. G. Jaurena is grateful for scholarships provided by the British Council and Fundación Antorchas (Argentina).

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## Integrated approach combining genetics, genomics and muscle biology to manage beef quality

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**Introduction** Up to now, genetic selection in cattle has been directed in favour of muscle growth, which changes muscle characteristics, and hence meat quality. One key concern, that now needs examination, is to understand the relationships between muscle growth and muscle characteristics related to meat quality. To achieve such a goal, muscles of divergently selected animals were analysed by three complementary approaches: (i) determination of muscle biochemical characteristics, (ii) identification of differentially expressed genes using transcriptomic and proteomic tools, (iii) identification of Single Nucleotide Polymorphisms (SNP) within candidate genes.

**Materials and Methods** Sixty four young Charolais bulls, progeny of 25 Charolais sires divergently selected for their muscle growth capacity index (a synthetic breeding value index for high muscle weight and low carcass fat percentage) were slaughtered at 15 or 19 months of age. Carcass composition was estimated from the 6<sup>th</sup> rib dissection. Animals were ranked on their own muscle growth capacity index and two groups of six bulls from each extremity of the index distribution were used in this study. Two muscle samples of *rectus abdominis* (red [RA]) and *semitendinosus* (white [ST]) were taken at slaughter. Four enzyme activities and collagen characteristics were determined as described (ref. in Hocquette *et al.*, *Recent Adv. Anim. Nutr. Aust.* 2001, 13, 135-143). Protease protein content was determined by Western Blot analysis and expressed relative to a control sample. Total RNAs were pooled in order to provide one sample for each muscle and each genetic type. Macro-arrays containing 1339 printed cDNA from a human muscle library were hybridised with radiolabelled complex cDNA from bovine mRNA (Piétu *et al.*, *Genome Res.* 1996, 6, 492-503). A bovine cDNA library was constructed starting from a pool of total RNA obtained from different muscle types. Glass slide arrays with a total of 480 bovine muscle cDNA probes were hybridised with fluorescently labelled total RNAs. For proteomic analysis, 50 µg of muscle protein were solubilised and separated by 2D-electrophoresis in a 4-7 pH gradient for the first dimension and by SDS-PAGE (11%) for the second dimension. Proteins were stained with silver nitrate. Differentially expressed proteins were identified by mass spectrometry. For each candidate gene studied, SNPs were identified by SSCP analysis and sequencing of PCR fragments.

**Results** Bulls with a high growth potential were characterised by a higher muscle mass (316.4 vs 249.2 kg) and a lower proportion of fat in the carcass (12.6 vs 17.4%) than bulls with a low growth potential ( $P < 0.01$ ). Metabolic enzyme activities and protease protein content significantly differed between RA and ST muscles, confirming the metabolic differences between the two muscle types (Table 1). A low growth potential was associated with lower proteasome 27K protein content and a more oxidative muscle metabolism, especially in RA muscle (Table 1). No significant differences were observed in collagen content or solubility between muscles or genetic types.

Table 1. Muscle characteristics of divergently selected bulls	High growth potential		Low growth potential		SEM	Significance*	
	RA	ST	RA	ST		Genetic type	Muscle
Isocitrate dehydrogenase activity	1.39 <sup>b</sup>	1.14 <sup>b</sup>	1.90 <sup>a</sup>	1.40 <sup>b</sup>	0.147	0.21	0.03
Citrate synthase activity	1.98 <sup>c</sup>	2.52 <sup>bc</sup>	3.93 <sup>a</sup>	3.35 <sup>ab</sup>	0.293	0.004	0.95
Lactate dehydrogenase activity	933 <sup>b</sup>	1176 <sup>a</sup>	900 <sup>b</sup>	1075 <sup>a</sup>	42.7	0.47	0.0006
µ-calpain protein content	113.6 <sup>b</sup>	122.2 <sup>ab</sup>	116.6 <sup>b</sup>	134.3 <sup>a</sup>	4.64	0.20	0.02
Proteasome 27K protein content	93.1 <sup>a</sup>	89.1 <sup>a</sup>	83.5 <sup>a</sup>	70.0 <sup>b</sup>	3.87	0.005	0.05

<sup>a,b,c</sup> Mean values within a row with different superscript letters differ significantly ( $P < 0.05$ ).

Transcriptome analysis, under heterologous conditions, on human macro-arrays has allowed the quantification of 375 gene prints out of the 1339 cDNA probes spotted. Thirty four genes were shown to be differentially expressed between both genetic types. Many of them are involved in muscle structure (*e.g.* titin and sarcosin) or in cellular regulation (*e.g.* thyroid hormone receptor interacting protein 10, heat shock protein 90a or LIM protein). Very recently, transcriptome analysis on glass slide bovine arrays allowed the analysis of 526 to 648 out of 1440 cDNA probes spotted (corresponding to 480 genes in triplicate). Most of the genes were not differentially expressed. Nevertheless, 4 of them presenting low differential expression are being characterised. In ST muscle of 15 month-old bulls, less than 5% of the separated proteins were differentially expressed in the two genetic types. They belong to different classes such as contractile or metabolic muscle characteristics. For example, troponin T slow isoform was less expressed, and myosin binding protein H was more expressed in ST from high growth potential bulls. Other proteins are currently being identified. SNP have been identified for several candidate genes; their relationship with other measured characteristics remains to be analysed. However, no SNP have been revealed yet in the gene encoding citrate synthase.

**Conclusions** Genetic selection for growth has an effect upon some muscle biochemical characteristics and changes the expression of several genes in muscles, which could have some impact on meat quality. By using the functional genomics approach (transcriptomic and proteomics analysis), new differentially expressed genes will be discovered, which may help scientists to identify new meat quality indicators. Combining the discovery of quantitative trait loci and polymorphisms, with gene expression profiling and classical muscle biochemistry in an integrative approach will bring about a better understanding of the relationship between muscle growth and meat quality traits.

# Genetic aspects of body weight, body condition score, scrotal circumference and reproductive performance in Italian Holstein young bulls

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**Introduction** Apart from all the issues regarding health and undesirable recessive genes, which are determining factors to identify bulls not suitable for AI, there are other aspects which can be considered. The principal aims of AI studs are the selection of the best animals from a genetic point of view and the production of semen in a very short time, in order to start progeny testing as soon as possible. In Italy progeny testing starts when 1200 straws for each young bull are available. This requirement implies that bulls should be in good physical condition. According to this, the genetic centre of the Italian Holstein Breeder Association (ANAFI) has been paying growing attention to animal welfare and fitness, recording routinely some biometrical indicators. Body weight (BW), scrotal circumference (SC), body condition score (BCS) are some examples of this policy and could be helpful and easy predictors of reproductive performance for a young progeny testing dairy bull. The objective of this report was to conduct a preliminary analysis on the genetic aspects of BW, BCS, SC, age at the 1<sup>st</sup> usable straw (AGE1) and age at the 1200<sup>th</sup> straw (AGE1200) in Italian Holstein young bulls.

**Materials and Methods** Data included measures of BCS, SC, BW, AGE1 and AGE1200 for 415 Holstein bulls, belonging to 4 AI studs and born from February 1997 to September 1999. Generally, young bulls enter the ANAFI Genetic Centre (AGCenter) at an average age of 220 d and undergo a 3-months health testing period. After a bull gets through all health tests he leaves the AGCenter and, on the same day, he goes back to the AI stud owner. Before leaving the AGCenter, traits as BCS, SC and BW are recorded. After a quarantine period at the stud, semen collection begins and the 1<sup>st</sup> usable straw is considered the first one containing 30 million spermatozoa with at least 10% motility. Genetic parameters for BW, BCS, SC, AGE1 and AGE1200 were estimated using a multi-trait animal model procedure. REML method using the VCE software (Groeneveld and Kovac, 1990) was applied to estimate variance components. The model for BW, BCS and SC included the fixed effects of year-season of arriving to the AGCenter, region, and age at measurement (linear covariate). The model for AGE1 and AGE1200 included the fixed effects of year-season of arriving to the AI stud, AI stud and age at measurement (linear covariate). A random animal effect was fitted in both models using the relationship matrix going back at least two generations (1850 animals).

**Results** Estimates of genetic and phenotypic parameters for BW, BCS, SC, AGE1 and AGE1200 are presented in Table 1. Heritability for BW and SC agree with estimates from other studies (Mohiuddin, 1993; Brinks, 1994). Estimate for BCS is lower than value found by Pryce *et al* (2000) in dairy cows. AGE1 presents a moderately high heritability, while AGE1200 results in smaller value due to a larger residual variance (690.73 vs 403.75). Genetic correlations between BCS and reproductive traits were moderately high, suggesting that fatter young bulls delay puberty or semen production. The relationship between SC, BW and reproductive traits were weak. However standard errors were high and correlations might not be fully informative and reliable.

**Table 1.** Unadjusted means, phenotypic standard deviation ( $\sigma_p$ ), heritability ( $h^2$ ), standard error (SE), residual (above diagonal) and genetic (below diagonal) correlations for BW, BCS, SC, AGE1 and AGE1200 (415 records).<sup>1</sup>

Trait	Mean	$\sigma_p$	$h^2$	SE	BW	BCS	SC	AGE1	AGE1200
BW, kg	353.46	41.12	0.304	0.092		0.130	0.46	-0.034	-0.224
BCS (1-5)	2.76	1.20	0.190	0.072	0.655		-0.082	-0.083	-0.098
SC, cm	32.61	1.26	0.174	0.062	0.228	0.764		-0.165	-0.218
AGE1, d	374.20	28.68	0.221	0.081	0.006	0.481	0.10		
AGE1200, d	414.76	30.96	0.107	0.057	-0.036	0.330	-0.025		

<sup>1</sup>SE of genetic correlations ranged from 0.129 to 0.245 while SE of residual correlations ranged from 0.052 to 0.073.

**Conclusions** These preliminary results supplied interesting information on the genetic aspects of BW, BCS, SC and reproductive performance in young progeny testing dairy bulls. Additive genetic variance is responsible for a certain proportion of the phenotypic variation in AGE1 and AGE1200. Moreover, the genetic correlations between AGE1 and AGE1200 and BCS seem to be not negligible. These relationships have positive implications for the evaluation of BCS as candidate predictor of reproductive performance. Furthermore, routine BCS assessment for young progeny test bulls can be performed at low cost by the AI stud's personnel. Nevertheless data were limited and in order to get more reliable estimates for genetic correlations, additional data or alternative statistical methods as Monte Carlo Markov Chain methodology should be used.

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# The genetic relationship between interval to commencement of luteal activity postpartum and UK national fertility proofs for dairy cattle.

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**Introduction** The coefficient of genetic variation of fertility traits is of a similar magnitude to that present in production traits, however traditional measurements of fertility have low heritability ( $h^2 < 0.05$ ), and recording is often poor, hindering the identification of genetically superior animals. The effect of sire on daughter fertility has been examined as part of a DEFRA LINK project to produce an UK fertility index. The project is investigating the use of six currently recorded traits to calculate sire genetic merit for fertility: calving interval (CI), interval to first service (DIMFIR), non-return rate 56 (NR56), number of services per conception (CINSOBS), milk yield and condition score (Wall et al., 2002). An alternative way to measure fertility is to use endocrine measurements such as interval to commencement of luteal activity postpartum (CLA). This parameter is less influenced by management decisions and has a moderate heritability (0.16; Royal et al., 2002a) and is measurable early in lactation. Although information on the genetic relationships between CLA and other traits of economic importance have been reported previously (Royal et al., 2002a; Royal et al., 2002b) further information would be useful in order to assess the usefulness of incorporating CLA into a future UK breeding programme. The objective of these analyses was therefore to obtain information on the genetic correlation ( $r_A$ ) between lnCLA and the emerging UK national fertility proofs.

**Materials and Methods** CLA, the fertility parameter analysed in this study is described in Royal et al 2000; Royal et al 2002a and Royal et al. (2002b). (Co)variance components for the four fertility index traits (CI, DIMFIR, CINSOBS and NR56) were estimated to calculate the genetic parameters using ASREML (Gilmour et al, 2000). These parameters were then used to estimate sire predicted transmitting abilities (PTAs) for each trait by multi-trait best linear unbiased prediction (BLUP) using PEST (Groneveld et al, 1990). Details of data restrictions and edits used during the estimation of (co)variance components and PTAs are described in full in Wall et al, 2002. Of the 169 sires in the milk progesterone database, 148 had proofs for fertility. A mixed linear model was fitted to the data using the restricted maximum likelihood method. ASREML (Gilmour et al, 2000) was used to estimate genetic regressions of lnCLA on each of the fertility traits by fitting the model with an additional term for the sire PTA.  $r_A$  were inferred from the regressions by multiplying the regression coefficient ( $b$ ) by the ratio of the genetic standard deviations (e.g. sire PTA/lnCLA).

**Results** Means, standard deviations, heritabilities, genetic regression coefficients ( $b$ ) of lnCLA on sire PTAs for each parameter, their standard errors and levels of significance, in addition to estimated  $r_A$  are presented in Table 1.

**Table 1** : Descriptive statistics for the regression of lnCLA on sire PTAs for fertility

Parameter	n	Mean	$h^2$	h	$\sigma_p$	$\sigma_A$	$b$	$b$ s.e.	P value	$r_A$ with lnCLA
LnCLA (days)	1212	29.4	0.16	0.40	0.52	0.208	-----	-----	-----	-----
CI (sire PTA; days)	148	386.2	0.04	0.21	46.67	4.794	0.017	0.00594	P<0.005	0.392
DIMFIR (sire PTA; days)	148	82.0	0.05	0.22	26.06	2.979	0.037	0.00932	P<0.0005	0.530
NR56 (sire PTA; 0, 1 or 2)	148	1.64	0.02	0.14	0.469	0.1	0.038	0.72600	n.s.	0.018
CINSOBS (sire PTA; 1-10)	148	1.65	0.03	0.17	0.975	0.1	0.099	0.43800	n.s.	0.048

The genetic regression coefficients ( $b$ ) of lnCLA on sire PTA for CI and DIMFIR were positive and significant. The inferred  $r_A$  were 0.39 and 0.53 respectively. These correlations indicate that cows with genetically longer CI and DIMFIR on average have a longer interval to CLA. The magnitude of the regression coefficients were such that CLA increased by 1.7% (0.43 days) with every day increase in CI and by 3.8% (0.96 days) with every day increase in DIMFIR. Genetic regressions of lnCLA on both NR56 and CINSOBS were not significantly different from zero.

**Conclusion** Since CLA has a moderate heritability and is measurable in all animals rather than only those that complete a lactation it may have the potential to help improve the accuracy of breeding value prediction for fertility if incorporated into fertility indices based on traditional fertility traits while using smaller progeny test groups.

**Acknowledgements** We thank DEFRA, NMR, HUK, Dartington Trust, CIS, Cogent and Genus for their support.

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## Genetic correlations among body condition score, body weight, and fertility in dairy cows

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**Introduction** Interest is accruing in indicator traits as predictors of fertility which: 1) can be more easily recorded; 2) can be measured early in life; and, 3) possess a co-heritability that is larger than the heritability of the fertility traits. Potentially interesting indicator traits include body condition score (BCS) and body weight (BW). The objective of this study was to estimate genetic (co) variances between BCS, BCS change, BW, BW change, and fertility traits in dairy cattle.

**Material and methods** The data analyzed included 8591 multiparous Holstein-Friesian cows with records for BCS, BW, and/or fertility from 78 seasonal calving grass based farms throughout southern Ireland. Of the cows included in the analysis, 4402 had repeated records across the two years of the study. Each cow had over three BCS and/or over three BW records per year. BCS and BW at day 5, 60, 120, 180 of lactation (CS5, CS60, CS120, CS180, BW5, BW60, BW120, BW180, respectively) were estimated for each cow as outlined by Berry *et al.*, (2002). BCS change and BW change from day 5 to day 60 of lactation (CS60-5 and BW60-5, respectively) were calculated as the difference between the traits at day 5 and day 60. In total 8315 cows had identified first service records. The four fertility variables were: calving to first service interval (CFS), pregnant to first service (PRFS), number of services per cow (NS), and pregnant by day 63 of the breeding season (PR63); their calculation is outlined in more detail by Evans *et al.*, (2002). A series of bivariate analyses were carried out in ASREML (Gilmour *et al.*, 2002) and the subsequent correlation matrix was made positive definite. The following linear animal model was used for the univariate and bivariate analysis of all fertility related traits:

$$Y_{ijkpq} = \mu_p + HYS_j + l_k + Hol_i + SOB_m + EOB_n + a_i + PE_i + e_{ijkpq}$$

Where:  $Y_{ijkpq}$  = observation for trait  $p$  on animal  $i$ ,  $\mu_p$  = overall mean for trait  $p$ ,  $HYS_j$  = herd by year by month of calving interaction,  $l_k$  = lactation number ( $k = 1, 2, 3, 4+$ ),  $Hol_i$  = quadratic polynomial regression for the percentage of North American Holstein-Friesian genes in animal  $i$ ,  $SOB_m$  = quadratic polynomial regression for the number of days between calving and start of the breeding season,  $EOB_n$  = quadratic polynomial regression for the number of days between calving and finish of the breeding season,  $a_i$  = random additive genetic effect,  $PE_i$  = random permanent environmental effect for animal  $i$ ,  $e_{ijkpq}$  = random residual term. The quadratic regression on calving to start of breeding and the quadratic regression on calving to end of breeding were only applied to the fertility traits.

**Results** Heritability estimates for the fertility traits were all less than 0.03, however a considerable genetic variation existed for some of these traits; PRFS showed a coefficient of genetic variation (CVg) of 11%. The CVg for BCS and BW were all less than 7%. Heritability estimates and genetic correlations between BCS, BCS change, BW, BW change, and the fertility traits are shown in Table 1. Based on the genetic parameters estimated in the present study between BCS and fertility, an increase in genetic merit for BCS at day 5 (CS5) of 1 BCS unit will reduce the CFS by 3 days, increase PRFS by 12 percentage units, reduce the NS by 0.4 and increase PR63 by 12 percentage units.

**Table 1.** Heritabilities and genetic correlations between BCS, BCS change, BW, BW change and fertility with their SE<sup>1</sup>.

Trait	CS5	CS60	CS120	CS180	CS60-5	BW5	BW60	BW120	BW180	BW60-5
$h^2$	0.29	0.43	0.43	0.41	0.07	0.39	0.53	0.57	0.45	0.06
CFS (Days)	-0.33 <sup>‡</sup>	-0.38 <sup>‡</sup>	-0.33 <sup>‡</sup>	-0.44 <sup>‡</sup>	0.06 <sup>§</sup>	-0.28 <sup>‡</sup>	-0.19 <sup>‡</sup>	-0.22 <sup>‡</sup>	-0.29 <sup>‡</sup>	0.28 <sup>§</sup>
PRFS (%)	0.46 <sup>°</sup>	0.28 <sup>°</sup>	0.34 <sup>°</sup>	0.26 <sup>°</sup>	-0.22 <sup>*</sup>	-0.11 <sup>°</sup>	-0.23 <sup>°</sup>	-0.26 <sup>°</sup>	-0.26 <sup>°</sup>	-0.26 <sup>°</sup>
NS (number)	-0.52 <sup>‡</sup>	-0.39 <sup>‡</sup>	-0.40 <sup>‡</sup>	-0.33 <sup>‡</sup>	0.17 <sup>°</sup>	0.07 <sup>‡</sup>	0.14 <sup>‡</sup>	0.19 <sup>‡</sup>	0.23 <sup>‡</sup>	0.26 <sup>°</sup>
PR63 (%)	0.41 <sup>§</sup>	0.29 <sup>‡</sup>	0.37 <sup>‡</sup>	0.39 <sup>‡</sup>	-0.19 <sup>°</sup>	-0.18 <sup>‡</sup>	-0.23 <sup>‡</sup>	-0.22 <sup>‡</sup>	-0.21 <sup>‡</sup>	-0.23 <sup>°</sup>

<sup>1</sup> <sup>†</sup> SE < 0.10; <sup>‡</sup> SE < 0.15; <sup>§</sup> SE < 0.20; <sup>°</sup> SE < 0.25; <sup>\*</sup> SE < 0.30; SE for the  $h^2$  were all < 0.06

**Conclusions** BCS was favourably correlated with improved fertility while BW exhibited negative genetic correlations with CFS, PRFS and PR63 and positive genetic correlations with NS. The co-heritability of BCS and BW with fertility was larger than the heritability for most of the individual fertility traits signifying that with small progeny group sizes faster rates of genetic improvement may be achieved through indirect selection for improved fertility.

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# Genetic parameters for locomotion and composite type traits for the Jersey and Guernsey dairy breeds in the United Kingdom (UK)

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**Introduction** There is growing interest in the dairy industry to broaden breeding objectives by incorporating health and welfare traits into selection indices. Although these traits are difficult to measure directly, there is good evidence to show that some linear type traits are genetically correlated with certain health and welfare traits, e.g. udder-type with somatic cell count (Mrode, Swanson and Lindberg, 1999) and locomotion with lameness (Boelling, 1996). Linear and composite type traits, such as locomotion and the feet and legs and mammary system composites, have been proposed for inclusion in a future modification to the UK Profitable Life Index (PLI). The objective of this study was to estimate genetic parameters of locomotion and composite traits for application in UK national dairy genetic evaluations for Jerseys and Guernseys.

**Materials and Methods** The data consisted of linear and composite type scores from the official classification schemes carried out by the Jersey and Guernsey Breed Societies. The traits included locomotion (**LOCO**), a linear type trait scored on a scale of 1 (poor) to 9 (very good), and body character (**BC**), dairy character (**DC**), feet and legs (**FL**) and mammary system (**UDD**), which are composite type traits scored on a scale of 40 (poor) to 89 (very good) (MDC Evaluations Ltd, 2002). Data used for analyses was restricted to classifications on heifers aged between 18 and 45 months and within 1 and 365 days of lactation. The data sets consisted of 6590 records for Jersey and 3838 records for Guernsey after edits. Multivariate analyses were carried out using ASREML software (Gilmour *et al.*, 2000) to obtain genetic parameter estimates. The animal model fitted included month of calving and herd-inspection date as fixed effects and age and stage of lactation at classification fitted as linear and quadratic covariables.

**Results** While the mean scores were higher in the Jersey breed, the lower sds reflected a more limited use of the scoring range. All heritabilities were moderate to moderately high in both breeds with the lowest found for FL and LOCO. Although the magnitude varied between the breeds, most genetic and phenotypic correlations were positive. The genetic relationships found between UDD, FL and LOCO were all moderate to strongly positive. A high genetic correlation of 0.91 was found in Guernseys between FL and LOCO. The same correlation in Jerseys was low at 0.30.

**Table 1.** Unadjusted means, standard deviations (sd) and parameter estimates<sup>1</sup> (s.e.)

Jersey							
	Mean	sd	BC	DC	FL	UDD	LOCO
BC	80.74	3.98	<b>0.25</b> (0.04)	0.29	0.30	0.25	0.18
DC	81.40	3.69	0.23 (0.09)	<b>0.36</b> (0.04)	0.18	0.28	0.12
FL	80.09	4.45	0.38 (0.11)	0.13 (0.11)	<b>0.19</b> (0.04)	0.22	0.32
UDD	79.18	5.27	0.56 (0.09)	0.25 (0.09)	0.41 (0.11)	<b>0.31</b> (0.04)	0.45
LOCO	5.38	1.17	0.15 (0.12)	0.04 (0.10)	0.30 (0.13)	0.63 (0.08)	<b>0.22</b> (0.04)
Guernsey							
	Mean	sd	BC	DC	FL	UDD	LOCO
BC	75.98	9.19	<b>0.51</b> (0.06)	0.54	0.15	0.28	0.12
DC	78.50	8.24	0.82 (0.04)	<b>0.41</b> (0.05)	0.22	0.43	0.16
FL	78.09	7.45	-0.09 (0.14)	0.08 (0.14)	<b>0.14</b> (0.04)	0.23	0.75
UDD	74.50	9.58	0.46 (0.09)	0.38 (0.10)	0.35 (0.14)	<b>0.29</b> (0.05)	0.23
LOCO	5.28	1.49	-0.02 (0.13)	0.03 (0.14)	0.91 (0.05)	0.42 (0.14)	<b>0.17</b> (0.04)

<sup>1</sup>Heritabilities on the diagonal (in bold), phenotypic correlations above diagonal and genetic correlations below diagonal

**Conclusions** Heritabilities found in this study suggest scope for genetic improvement for all traits considered. The heritability estimates for composite traits were in reasonable agreement to those found in Holstein-Friesians (HF), although estimates found for LOCO in this study were slightly higher ( $h^2=0.11$  in HF) (S.Brotherstone, pers.com.). The positive correlations between the feet (FL and LOCO) and mammary (UDD) traits is promising for inclusion of these traits in a revised PLI for Jerseys and supports the inclusion of FL and UDD in the Guernsey Merit Index already in use. Locomotion is widely considered as a more objective measure than the feet and leg composite and since these traits are highly correlated in Guernseys, consideration should be given to replacing the feet and leg composite with locomotion. The unexpectedly low correlation between these traits in the Jersey breed requires further investigation.

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## The influence of cow genetic merit for milk production on response to level of concentrate supplementation in a grass based system.

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**Introduction** Pre-1990 published responses to supplementation at pasture ranged from 0.4 to 0.6kg milk/kg concentrate fed. However since 1990 higher responses to concentrate supplementation at pasture have been published (Delaby 2001). The objective of this study was to determine if milk production responses of Holstein-Friesian dairy cows to concentrate supplementation at pasture are influenced by genetic merit (milk yield potential) in a spring calving grass based system of milk production.

**Materials and Methods** The study was carried out at Moorepark Research Centre, between January 1998 and December 2000. A total of 48 high genetic merit (HM) and 48 medium genetic merit (MM) cows were randomly assigned to the three concentrate feeding levels. Mean pedigree index (and s.d.) within each group were +276 kg milk (100), +8.9 kg fat (4.75), +9.7 kg protein (3.19), -0.03 g fat/kg (0.086) and +0.01 g protein/kg (0.035) for the HM group; the corresponding values for the MM group were +81 kg milk (95), +3.8 kg fat (4.95), +4.3 kg protein (2.59), +0.013 g fat/kg (0.099) and +0.031 g protein/kg (0.036). All the animals were first lactation animals in 1998. A total of 66 cows remained in the study in the same feeding system for the three-year duration of the study. In early May cows were grouped into blocks of three within genotype, on the basis of calving date and milk yield, and randomly assigned to one of three concentrate feeding levels. Cows remained on these feeding systems for the duration of the trial. The low concentrate (LC), medium concentrate (MC), and high concentrate (HC) feeding systems were allocated 376, 810 and 1540 kg/cow/lactation. The aim was for similar grazing management across all three concentrate feeding levels. In both 1999 and 2000 concentrate-feeding levels were imposed on all animals immediately post calving. Milk yields were recorded daily, while concentration of fat, protein and lactose were determined in one successive morning and evening sample/week. The data was first analysed as a split plot design with genotype as the main plot and concentrate feeding level as the sub plot. Secondly the data was analysed for individual cow performance using covariates pre experimental milk and protein yield (experimental weeks 17 and 18 in 1998), and pedigree index (PD00) for milk and protein yield analysis.

**Results** Treatment comparison analysis showed a significant effect of genotype and concentrate feeding level for yield of milk, solids corrected milk yield (SCM) (Tyrell and Reid, 1965), fat, protein and lactose (P<0.001). There was a significant genotype by concentrate feeding level interaction (G\*F) for fat yield and milk fat concentration while SCM approached significance (P=0.07).

**Table 1** The average effect of genotype and concentrate feeding level on milk production 1998-2000.

Genotype	MM†			HM‡			Genotype		Feed		G x F
	LC	MC	HC	LC	MC	HC	SE	Sig.	SE	Sig.	
Milk (kg/cow)	6421	6681	7196	7389	7739	8461	95.1	***	58.8	***	NS
SCM (kg/cow)	5938	6380	6674	6683	7013	7666	72.7	***	54.6	***	†
Fat (kg/cow)	247	269	272	274	288	313	2.9	***	2.8	***	*
Protein (kg/cow)	217	232	250	247	261	288	2.8	***	2.1	***	NS
Lactose (kg/cow)	299	312	339	343	357	391	4.4	***	2.8	***	NS
Fat (g/kg)	38.6	40.6	38.0	37.2	37.3	37.2	0.30	***	0.34	***	*
Protein (g/kg)	34.0	34.8	34.9	33.5	33.8	34.1	0.15	**	0.16	***	NS
Lactose (g/kg)	46.6	46.8	47.2	46.4	46.1	46.2	0.12	NS	0.11	***	†

Covariance analysis showed a significant interaction (P<0.05) between level of concentrate supplementation and both pedigree index (PD00) for milk and protein yield (used as a covariable). Using pre-experimental milk yield and milk protein yield the interaction was approaching significance (P=0.08 for milk yield and P=0.09 for protein yield).

**Conclusions** The present experiment was designed to examine whether the production responses to supplementary concentrate, given in an adequate grass supply situation, are influenced by the genetic merit of the dairy cow. The results suggest that there is a genotype x concentrate feeding level interaction for milk production. This implies that the value of increased genetic merit in a low concentrate grass - based system of milk production will be less than in a high concentrate system and that the response to increased concentrate feeding depends on the genotype of the animal.

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## Evaluation of dual-purpose cows on a seasonal grass-based system of milk production.

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**Introduction** In recent years the relevance of continued selection for higher milk yield alone has been questioned for three reasons (Simm, 1998): 1) the introduction of milk quotas in some countries, 2) the now well documented deleterious effect of selection for yield on health and fertility (Pryce and Veerkamp, 2001), and, 3) the increased emphasis in payment schemes in many countries on milk composition. The purpose of this study was to measure the biological efficiency of two dual-purpose breeds (Montbeliarde (MB) and Normande (NR)) relative to two Holstein-Friesian strains (upgraded Irish Holstein-Friesian (CL) and Dutch Holstein-Friesian (HF)) on a spring-calving milk production system based mainly on grazed grass as a feed.

**Material and methods** A total of 116, 100, 112, 108 and 112 animals were allocated to the trial in 1996, 1997, 1998, 1999 and 2000 respectively, divided equally between the four breeds. Twenty, 9, 11 and 12 different sires were represented in the HF, CL, MB and NR groups, respectively. The breeds were balanced for calving date and parity in mid-April of each year. Cows were managed on a rotational grazing system (Dillon *et al.*, 1995), with concentrate supplementation averaging 644 kg DM per cow per year. Individual cow milk yield and composition was recorded on one day per week. Live weight (LW) was recorded once every two weeks and body condition score (BCS) (scale 1 to 5) every 3-4 weeks. The breeding period was 14 weeks, starting in late April. Reproductive parameters calculated included submission rate in the first 24 days of the mating period, pregnancy rate to first service and overall pregnancy rate, calving to service interval (CSI), calving to conception interval (CCI) and no. of services per conception. Data for milk production, BCS, CSI and CCI were analysed using the general linear model procedure of SAS (1991). Between year variation was not significant ( $P>0.05$ ); therefore, data were pooled across years.

**Results** The HF cows produced the highest ( $P<0.05$ ) yield of milk, fat, protein and lactose; the NR produced the lowest, while the CL and MB were intermediate (Table 1). The NR produced the highest ( $P<0.05$ ) milk fat, protein and lactose content. The HF had significantly lower LW gain during lactation. Both the HF and CL had lower ( $P<0.05$ ) BCS at all stages of lactation than the MB and NR. The HF had significantly greater BCS loss over the first 8 weeks of lactation compared to the other three breeds. After 14 weeks breeding, significantly more HF cows (26.3%) were not pregnant compared to the CL cows (16.1%); both of these empty rates were higher than the MB (8.8%) and NR (8.1%) breeds. Furthermore, the HF cows had a greater CCI than the other three breeds ( $P<0.05$ ).

**Table 1** The effect of dairy cow breed on performance parameters over five years

	Breed of cow				<sup>1</sup> SED	Significance
	HF	CL	MB	NR		
Lactation length (days)	303	301	298	301	3.4	ns
Milk (kg/cow)	5,994 <sup>a</sup>	5,321 <sup>b</sup>	5,119 <sup>b</sup>	4,561 <sup>c</sup>	119.8	***
Fat (g/kg)	39.0 <sup>a</sup>	37.5 <sup>b</sup>	38.1 <sup>b</sup>	40.0 <sup>c</sup>	0.47	***
Protein (g/kg)	33.9 <sup>a</sup>	33.6 <sup>a</sup>	34.9 <sup>b</sup>	36.0 <sup>c</sup>	0.25	***
Lactose (g/kg)	46.2 <sup>a</sup>	46.2 <sup>a</sup>	47.3 <sup>b</sup>	47.9 <sup>c</sup>	0.21	***
Pre-calving BCS	3.04 <sup>a</sup>	3.03 <sup>a</sup>	3.34 <sup>b</sup>	3.32 <sup>b</sup>	0.058	***
BCS week 8 of lactation	2.55 <sup>a</sup>	2.72 <sup>b</sup>	3.00 <sup>c</sup>	3.04 <sup>c</sup>	0.054	***
BCS end of lactation	2.45 <sup>a</sup>	2.81 <sup>b</sup>	3.11 <sup>c</sup>	3.11 <sup>c</sup>	0.064	***
Submitted during the 1 <sup>st</sup> 24 days of breeding (%)	75.2 <sup>b</sup>	83.9 <sup>ab</sup>	87.6 <sup>a</sup>	83.8 <sup>ab</sup>	-	*
Pregnancy rate to first service (%)	37.2 <sup>c</sup>	42.3 <sup>bc</sup>	50.4 <sup>ab</sup>	56.6 <sup>a</sup>	-	**
Overall pregnancy rate (%)	73.7 <sup>c</sup>	83.9 <sup>b</sup>	91.2 <sup>a</sup>	91.9 <sup>a</sup>	-	***
Calving to service interval (days)	71.5 <sup>a</sup>	70.9 <sup>ab</sup>	64.9 <sup>b</sup>	67.7 <sup>ab</sup>	2.38	*
Calving to conception interval (days)	99 <sup>a</sup>	87.3 <sup>b</sup>	82.1 <sup>b</sup>	82.9 <sup>b</sup>	3.16	*
Services/pregnant cow (no.)	2.79 <sup>a</sup>	2.39 <sup>a</sup>	1.99 <sup>b</sup>	1.82 <sup>b</sup>	-	**

<sup>1</sup>SED = standard error of difference, ns = not significant ( $P>0.05$ ); \* $P<0.05$ ; \*\* $P<0.01$ ; \*\*\* $P<0.001$

**Conclusions** The results of this study suggest that although the HF produced the highest milk production, much of this was achieved through greater mobilisation of body reserves in early lactation and lower live weight gain from mid- to end of lactation. The results also indicate that the reproductive performance of HF with a large proportion of North American genes is low in a seasonal grass-based milk production system.

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# Effect of digestible undegradable protein (DUP) concentration of concentrates offered to ewes on grass-based diets in late pregnancy on colostrum production and lamb performance

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**Introduction** Nutrition of ewes during late pregnancy is a key factor influencing lamb survival and subsequent lamb growth and performance. Results from on-farm trials indicate that the superior lamb output from highly prolific compared with moderately prolific ewe breed types is less in grass-based compared with indoor lambing systems (Carson and Dawson, 2002). This is as a result of higher mortality rates of triplet lambs in outdoor systems with lower levels of intervention. Feeding and management strategies to maximise lamb viability for multiple births are required for grass-based lambing systems. Protein nutrition is likely to be a key factor, particularly considering that energy intakes in late pregnancy in triplet-bearing ewes are unlikely to meet requirements (Robinson, 1983). At sub-optimal energy intakes the supply of rumen undegraded protein is a major determinant of colostrum production and lamb birth weight. The objectives of this experiment were to investigate the effect of supplementing grass-based diets with concentrates with a range of digestible undegradable protein concentrations on colostrum production and lamb output.

**Materials and methods** Six weeks prior to lambing, sixty triplet-bearing ewes (liveweight  $84 \pm 8.4$  kg; condition score  $3.8 \pm 0.3$ ) of mixed breeds (Greyface; Texel X Greyface and Rouge X Greyface) mated to Texel sires were individually housed and allocated to one of five treatments (n=12) on the basis of condition score. Treatments 1-4 were offered fresh grass daily (0.6 kg DM/d) along with one of four isoenergetic and isonitrogenous (13.2 MJ ME/kg DM; 204 g CP/kg DM) supplements (0.55 kg DM/d) containing barley and varying proportions of xylose-treated soyabean meal and urea. The concentrates were formulated to supply 12; 25; 50 and 65 g DUP/d for treatments 1-4 respectively. Control ewes were offered an unsupplemented grass diet of equivalent total dry-matter allowance (1.15 kg DM/d). Fresh grass was harvested thrice weekly using a double-chop harvester and offered daily as a single feed at 0930 h while concentrates were fed in three equal sized meals at 0930, 1300 and 1630 h. Intakes of fresh grass and concentrates were recorded daily. Ewes were blood sampled at 6, 4 and 2 weeks pre-lambing and ewe liveweights and condition scores were recorded at 6, 4 and 2 weeks pre-lambing, at lambing, 6 weeks post-lambing and at weaning. Colostrum yield was determined by hand milking at 1, 10 and 18 h post-lambing, following intramuscular administration of 10 i.u. oxytocin. Lambs were weighed within 1 h of birth and fortnightly thereafter until weaning. Ewes and lambs were returned to pasture within 48 h of lambing. Data were analysed using REML (Restricted Maximum Likelihood) analysis with fixed effects for treatment, ewe breed and treatment x ewe breed.

**Results** Increasing the DUP concentration of the concentrate had no effect on pre-partum liveweight or body condition score change, total lamb birth weight or plasma BHB concentration. In all treatments pre-partum plasma BHB concentration increased significantly ( $P < 0.001$ ) towards term. Pre-partum plasma NEFA concentration was significantly ( $P < 0.001$ ) reduced by concentrate supplementation, and increased ( $P < 0.001$ ) towards term in all treatments. Total colostrum yield was unaffected by dietary treatment, although unsupplemented control ewes tended to have the lowest colostrum yields at each milking interval. Increasing the DUP concentration of the concentrate tended ( $P = 0.08$ ) to increase lamb survival to 6 weeks and led to a significant increase ( $P < 0.05$ ) in lamb survival to weaning. Dietary treatment had no effect on the total weight of lamb weaned per ewe.

**Table 1.** Effect of concentrate DUP concentration on colostrum production and lamb output

	Dietary Treatment					s.e.d	Sig
	Control	1	2	3	4		
†Pre-partum LW change (g/d)	284	298	274	297	337	37.2	NS
†Pre-partum BCS change	-0.15	-0.01	-0.13	-0.13	-0.05	0.10	NS
†Mean pre-partum BHB conc. (mmol/l)	1.28	0.79	0.88	1.01	0.95	0.22	NS
†Mean pre-partum NEFA conc. (meq/l)	0.74 <sup>b</sup>	0.51 <sup>a</sup>	0.55 <sup>a</sup>	0.56 <sup>a</sup>	0.53 <sup>a</sup>	0.058	***
Colostrum yield (g)	1514	2092	1828	2190	1938	341.7	NS
Total lamb birth weight (kg)	11.42	12.28	11.79	11.80	11.53	0.58	NS
Lambs weaned/ewe	2.00 <sup>ab</sup>	1.89 <sup>a</sup>	2.55 <sup>bc</sup>	2.56 <sup>bc</sup>	2.75 <sup>c</sup>	0.30	*
Weight of lamb weaned (kg/ewe)	66.3	62.4	77.6	81.0	77.5	8.79	NS

† Pre-partum period from 6 weeks to 2 weeks prior to the predicted mean lambing date

**Conclusions** For concentrates which are isoenergetic and isonitrogenous, increasing the level of DUP in the concentrate offered to triplet-bearing ewes on grass-based diets for the final six weeks of pregnancy has no effect on colostrum yield or total lamb birth weight, but leads to significant improvements in post-natal lamb survival.

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## The effect of maternal undernutrition on muscle fibre type in the newborn lamb

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**Introduction** Muscle fibre type can influence meat quality (Maltin *et al* 1997). Muscle fibre formation occurs during gestation and in the sheep the total number of fibres in a muscle is essentially fixed at birth. (Ashmere *et al* 1972). Postnatal growth of muscle is entirely due to elongation and widening of the existing muscle fibres. Therefore the gestational period is important in the long-term growth potential of the animal. By investigating changes in muscle fibre type, the aim of this study was to test the general hypothesis that the poor carcass quality sometimes seen in ruminant animals may be due to poor nutrition at strategic time points during the animal's development. As agricultural practices continue to become more extensive, variation in the nutrient supply to the animal is becoming more common. Therefore it is important to understand the effect of any changes in nutrient supply to the mother, during gestation on the subsequent muscle development of the fetus and ultimately the effects on meat quality.

**Materials and methods** 32 pregnant ewes (North Country mules) carrying twins were used in this study. The ewes were mated naturally and checked every 2 days. Day zero of gestation was taken as the first day at which the ewes had an obvious raddle mark. At day 20 the ewes were individually housed and fed a pelleted diet consisting of straw nuts and soya. The amount given was calculated on an individual ewe basis to provide 100% of their daily maintenance (M) requirement (AFRC 1993). The diet was fed in two equal rations and supplied 8.6MJ/day at the start. The ewes were randomly allocated into one of four groups (n=8). Group d30-d70 ewes were fed M diet until d30, the diet was then dropped to 50% M until d70, they then returned to 100% M until term. Group d55-95 ewes were similarly restricted from d55-d95, group d85-115 ewes were restricted from d85-d115. Group 4 ewes were the control group and were fed 100% M throughout gestation. The time periods of restriction were allocated based on previous studies identifying when muscle differentiation occurs (Fahey *et al* 2003). After parturition the ewes were fed a normal commercial diet for the lactating ewe, calculated on an individual ewe basis according to their live weight, on day 14 (after parturition) the lambs were euthanised by an overdose of pentobarbitone sodium Ph.Eur (1.33 ml/1kg body wt). Samples (10-20g) of the *Longissimus Dorsi* (LD), *Semitenidosus* (ST), and the *Vastus Lateralis* (VL), were dissected and were snap frozen in liquid nitrogen. The Immunochemical determination of myosin heavy chain slow (MHCs), myosin heavy chain fast (MHCf) and actin (as a control) proteins was measured by immunoprobings of Western blots using isoform specific monoclonal antibodies. These data were analysed using ANOVA using the Tukey test as a post hoc analysis.

**Results** Group d30-d70 expressed significantly less MHCf protein than the other treatment groups, in both the VL ( $p<0.05$ ) (\* = d30-d70 compared to all other treatment groups  $p<0.05$ ) and LD ( $p<0.005$ ) (figure 1) (\* = d30-d70 compared to all other treatment groups  $p<0.05$ ). The opposite was observed with MHCs as group 1 d30-d70 expressed more MHCs protein in both VL ( $p<0.001$ ) (\* = d30-70 compared to d85-d115 and control  $p<0.005$ ) and LD ( $p<0.05$ ) (figure 2) (\* = d30-d70 compared to all other treatment groups  $p<0.05$ ). No significant change was observed with ST.

Figure 1: The expression of MHCf in VL and LD.

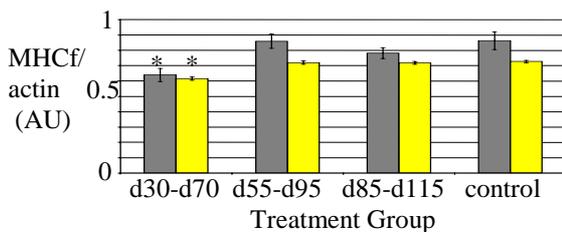
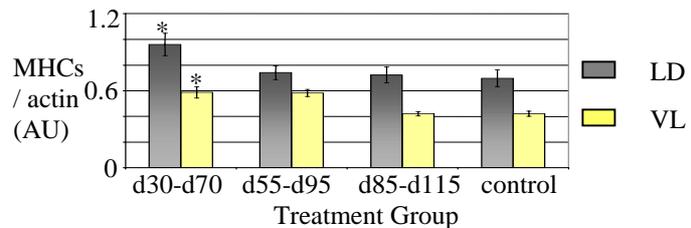


Figure 2: The expression of MHCs in VL and LD



**Conclusion** Primary muscle fibres tend to form the type I slow oxidative fibres in later life, whereas secondary fibres tend to form the type II fast fibres. This study indicates a reduction in the number of fast muscle fibres (secondary fibres) in the lambs born from the ewes d30-d70. This suggests that muscle fibre number is reduced due to maternal under-nutrition, but that only the secondary fibres are affected, while primary fibres appear resistant to manipulation. Although the two proteins in this study cannot be directly compared, we suggest that the increase seen in MHCs in the lambs born from the ewes d30-d70 is due to the change in proportion of fibres, i.e. there are not more slow fibres but just proportionally more in the sample due to the absence of secondary fibres. The results show that there is a time period during gestation where ovine muscle development appears to be manipulated by nutrition. It is yet to be seen whether these changes effect meat quality.

**Acknowledgements** A.J.Fahey was supported by a BBSRC studentship.

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# The effect of vitamin E and long-chain polyunsaturated fatty acid supplementation of pregnant and lactating ewes on the transfer of vitamin E to the lamb

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**Introduction** It is reported that supplementing pregnant ewes with supra-optimal levels of vitamin E improves neonatal lamb vigour and growth rate (Merrell, 1998). The biochemical mechanism behind these observations has yet to be elucidated as several studies report negligible placental vitamin E transfer in ruminants (Van Saun *et al.*, 1989); consequently, lambs may be clinically deficient in this nutrient at birth and achieve a satisfactory vitamin E status via colostrum ingestion. Lamb vitamin E status may be further diminished by the addition of polyunsaturated fatty acids (PUFAs) to the maternal diet. However, PUFA supplementation demonstrably enhances foetal and neonatal development in human studies (Morley, 1998) although these effects have not been investigated in ruminants to any depth. The objective of this experiment was to investigate the effects of dietary vitamin E in combination with long-chain PUFA 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 Megalac (M) or fish oil plus Incromega (a source of docosahexaenoic acid fed at a 25:75 ratio with fish oil) (F) as the main fat source and a basal (B; 50mg/kg) or supra-optimal (S; 500mg/kg) concentration of vitamin E. The concentrates were isoenergetic, isonitrogenous and formulated to provide 80g fatty acids/kg DM. Straw was offered *ad-libitum*. Twelve triplet lambs were euthanased immediately after birth to provide brain and muscle (*Longissimus dorsi*) samples and blood samples were obtained via cardiac puncture after cessation of the heartbeat. Lamb birthweight data was recorded at 12 hours *post partum*. Blood samples were taken from lambs by jugular venepuncture at 14 days of age and analysed for vitamin E, creatine kinase (CK) and glutathione peroxidase (GSHPx). Colostrum and milk samples were obtained from ewes at +16 hours and 21 days *post partum*. Vitamin E analysis was performed by HPLC. Data were analysed by ANOVA.

**Results** Maternal supra-optimal vitamin E supplementation significantly increased brain (P<0.05) and muscle (P<0.01) vitamin E concentrations in neonatal lambs. However, vitamin E was only detectable in 3 of 12 neonatal plasma samples. Supra-optimal vitamin E supplementation also significantly increased lamb birthweight (P<0.05). Vitamin E concentrations in colostrum, milk and lamb plasma were significantly augmented by supplementation (P<0.001) but reduced by dietary PUFA supplementation (P<0.001, P<0.001 and P<0.01 respectively). There was no effect of vitamin E on indicators of selenium status (GSHPx) or tissue damage (CK); however, PUFA supplementation significantly reduced erythrocyte GSHPx and increased serum CK concentrations.

**Table 1** Effects of supplementing pregnant and lactating ewes with vitamin E and long-chain polyunsaturated fatty acids on neonatal and growing lamb vitamin E status

	Treatment Diet <sup>#</sup>				s.e.d	Significance		
	MB	MS	FB	FS		Fish	Vitamin E	Interaction
Neonatal brain vitamin E (:g/g)	1.59	2.85	1.57	2.11	0.389	NS	*	NS
Neonatal <i>L. dorsi</i> vitamin E (:g/g)	0.69	1.18	0.57	0.95	0.127	NS	**	NS
Lamb birthweight (kg)	3.87	4.01	3.85	4.33	0.190	NS	*	NS
Colostrum vitamin E (:g/g) <sup>¶</sup>	6.0	28.2	3.7	8.2	0.346	***	***	***
Milk vitamin E (:g/g) <sup>¶</sup>	0.96	3.44	0.65	1.96	1.386	***	***	NS
Plasma vitamin E (:mol/l)	2.47	7.48	1.60	4.02	0.816	**	***	*
Serum CK (U/l)	168.3	144.9	437.6	436.2	115.00	*	NS	NS
Erythrocyte GSHPx (U/ml PCV)	289.2	308.0	273.4	277.1	16.33	*	NS	NS

<sup>#</sup>MB = Megalac + 50mg/kg vitamin E; MS = Megalac + 500mg/kg vitamin E; FB = Fish + 50mg/kg vitamin E; FS = Fish + 500mg/kg vitamin E <sup>¶</sup>Original means stated plus s.e.d for skewed data transformed by  $x^{0.5}$

**Conclusions** This study demonstrates that vitamin E deposition in neonatal lamb tissues may be manipulated via the maternal diet, although neonatal plasma concentrations are unaffected by supplementation. Moreover, lamb birthweight is increased by supra-optimal supplementation of pregnant ewes and the vitamin E status of growing lambs is augmented by mammary transfer into colostrum and milk. Maternal PUFA supplementation tends to reduce lamb vitamin E status.

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# Effects of diet and vitamin E supplementation on the distribution of vitamin E in plasma, muscle, liver and adipose tissue in lambs fed on concentrates alone or with grass silage

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**Introduction** Vitamin E protects the animal from oxidative stress *in vivo* and when it is administered at supranutritional levels it enhances the oxidative stability of meat. Studies have shown that poor absorption of vitamin E often occurs when concentrates are fed to lambs. Distribution of vitamin E in plasma, skeletal muscle, liver (short-term and fast release depot) and adipose tissue (long-term and slow release depot) provides useful information about the overall vitamin E status of the animals. This work studied the distribution of vitamin E in the tissues after supplementation in relation to diet and to the dietary level.

**Materials and Methods** Seven groups of eight Suffolk × Charollais wether lambs which came off grass, were individually penned and allocated by live weight to one of two diets (1) concentrates *ad libitum*, (2) grass silage (first cut rye grass) *ad libitum* plus 400g/day concentrates (mixed diet) for two months. The concentrate consisted of wheat, soya hulls, soya bean meal, rape seed meal, oatfeed and molasses. The concentrate based diets were supplemented with 30, 60, 120, 250 or 500 and the mixed diets were supplemented with 60 or 500 mg *all-rac-α*-tocopheryl acetate/kg DM. To obtain slaughter weights of 40kg lambs fed on concentrates alone entered the trial at a live weight of 24.8 ± 1.6 kg (SD) and lambs on the mixed diet started at a live weight of 32 ± 3.3kg (SD). Slaughter samples of plasma, *m. semimembranosus*, liver and subcutaneous adipose tissue (tail head fat) were collected for vitamin E analysis by HPLC. Data were analysed by ANOVA using diet and vitamin E supplementation level as fixed factors (SPSS 10.0.5. 1999).

**Results** Lambs fed on concentrates and supplemented with vitamin E at 250mg/kg DM or lower had plasma vitamin E levels similar to those associated with clinical deficiency. Tissue vitamin E levels in the concentrate fed lambs were in the order: plasma < muscle < liver < adipose tissue which is observed when the dietary vitamin E intake is very low instead of the normal order of: plasma < muscle < adipose tissue < liver (Table 1 and Figure 1). High plasma vitamin E levels such as in the concentrate fed lambs on the 500mg supplement did not ensure optimum vitamin E levels in the liver. The normal distribution pattern was barely maintained in the mixed diet fed lambs on the 60mg supplement. Inclusion of grass silage resulted in higher tissue vitamin E levels at the same supplementation level. Tissue vitamin E increased according to the dietary level. There were no significant interactions between diet and vitamin E level in any of the tissues except for adipose tissue (Table 2).

Table 1. Plasma, muscle, adipose tissue and liver vitamin E levels at slaughter

Tissue	30 (C)	60 (C)	120 (C)	250 (C)	500 (C)	60 (M)	500 (M)	sed	significance
Plasma (µg/ml)	0.07 <sup>a</sup>	0.22 <sup>ab</sup>	0.40 <sup>abc</sup>	0.76 <sup>bc</sup>	1.57 <sup>de</sup>	0.98 <sup>cd</sup>	1.93 <sup>e</sup>	0.204	***
Muscle (µg/g)	0.73 <sup>a</sup>	1.11 <sup>a</sup>	1.52 <sup>a</sup>	2.55 <sup>b</sup>	3.73 <sup>cd</sup>	3.15 <sup>bc</sup>	5.12 <sup>e</sup>	0.333	***
Adipose tissue (µg/g)	1.54 <sup>a</sup>	2.55 <sup>a</sup>	4.40 <sup>abc</sup>	7.35 <sup>bc</sup>	15.56 <sup>d</sup>	7.81 <sup>c</sup>	15.48 <sup>d</sup>	1.515	***
Liver (µg/g)	0.55 <sup>a</sup>	1.54 <sup>a</sup>	2.77 <sup>ab</sup>	6.09 <sup>bc</sup>	11.33 <sup>cd</sup>	7.80 <sup>c</sup>	18.73 <sup>e</sup>	1.205	***

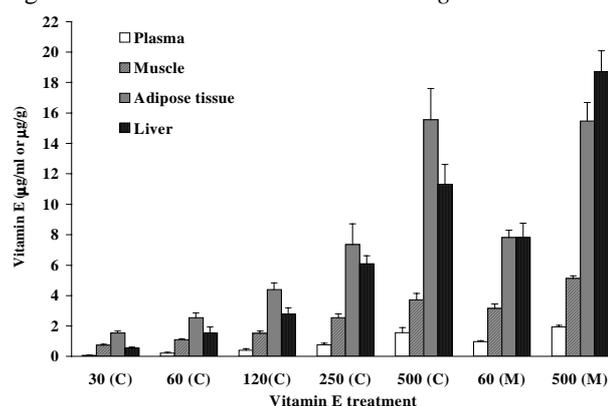
\*\*\*p<0.001 <sup>a, b, c, d, e</sup> means with a different superscript differ significantly at p<0.05

Table 2. Diet, vitamin E level or combination of diet plus vitamin E level effects in tissue vitamin E levels for treatments 60 (C), 500 (C), 60 (M) and 500 (M)

Tissue	Diet	Vitamin E	Diet × Vit. E
Plasma	**	***	NS
Muscle	***	***	NS
Adipose tissue	*	***	*
Liver	***	***	NS

\*p<0.05; \*\*p<0.01; \*\*\*p<0.001; NS not significant

Figure 1. Tissue vitamin E levels at slaughter



**Conclusions** Adequate plasma vitamin E levels may disguise sub-optimal levels in the tissues. Changes in the tissue vitamin E distribution pattern are related to changes in the overall vitamin E status of the animal as also shown by Jensen *et al.* (1990). The lack of interaction between diet and dietary vitamin E level indicates that low tissue vitamin E levels are probably due to poor absorption. Grass silage helps towards a better absorption.

**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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## Effect of herbage allowance and concentrate feed level offered to ewes in late pregnancy on ewe and lamb performance

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**Introduction** Lower cost systems of sheep production, with reduced labour inputs, require examination for the future viability of the sheep industry. Carson and Dawson (2002) demonstrated that ewes lambing under grass-based systems had similar lamb outputs and survival rates to ewes lambing under labour intensive, indoor lambing systems. Although there is a significant amount of information available on the effect of conserved forage on ewe performance in late pregnancy, there is a scarcity of information on the effect of grazed grass and concentrate supplementation at grass in late pregnancy on subsequent ewe performance. The aims of this study were to investigate the effects of herbage allowance and concentrate feed level during late pregnancy on ewe live weight and body condition change and lamb growth and development.

**Materials and methods** Six weeks prior to lambing, ninety six twin-bearing Greyface (Border Leicester X Scottish Blackface) ewes (liveweight  $73 \pm 11.2$  kg; condition score  $3.4 \pm 0.5$ ) mated to Texel sires were allocated to eight treatments (n=12 per treatment) on the basis of condition score. The treatments comprised two herbage allowances - low (LOW) (1.3 kg DM/day) or high (HIGH) (2.6 kg DM/day). Within the low herbage allowance 250, 500, 700 or 1000 g concentrates fresh were offered per ewe per day and within the high herbage allowance 0, 250, 500 or 750 g concentrates fresh were offered per ewe per day. Ewes were grazed in plots with 6 ewes per plot and were allocated fresh grass daily. A regression relationship between extended tiller height and herbage mass was produced and from this the area required to provide high or low herbage allowance was determined. Concentrates were offered in two equal sized meals at 0900 and 1600 h. Dry matter intakes were determined using the n-alkane method (Mayes *et al.* 1986). Ewe liveweight and condition score were recorded weekly and ewes were blood sampled every two weeks. Lambs were weighed at birth and fortnightly until weaning. Ewes lambed at grass after which ewes and lambs were moved to post-lambing fields. The 8 treatments were analysed as a 2 herbage X 3 concentrate factorial plus 2 extra treatments with regression contrasts calculated to test for linear effects.

**Results** Ewe body condition score increased with increasing concentrate supplementation (LOW,  $P=0.09$ ; HIGH,  $P<0.05$ ). Grass DM intakes indicated utilisation rates of total herbage offered from 28% for the high herbage allowance to 47% for the low herbage allowance. Increasing concentrate supplementation of ewes on the LOW herbage allowance up to 500 g/day had no effect on herbage intake, but concentrate supplementation at levels above 750 g/day decreased herbage intake. On the HIGH herbage allowance, herbage DM intake decreased by 20 g/day for each 100 g increase in concentrate DM intake ( $P<0.05$ ). Ewes on the LOW herbage allowance tended to have higher concentrations of BHB ( $P=0.05$ ) than those on the HIGH herbage allowance. Lamb birth weight and lamb live weight gain from birth to weaning were not significantly affected by concentrate supplementation on either the HIGH or LOW herbage allowance.

**Table 1.** Effect of herbage allowance and concentrate feed rate on ewe and lamb performance

Herbage allowance (H)	Concentrate feed rate (g fresh/day) (C)	Ewe liveweight post-lambing (kg)	Ewe condition score post-lambing	Ewe LW change (kg) <sup>#</sup>	Herbage DM intake (g/day) <sup>†</sup>	Ewe BHB (mmol/l) <sup>‡</sup>	Lamb performance	
							Birth weight (kg)	Liveweight gain (birth to weaning) (g/day)
LOW (1.3 kg DM/day)	250	67	3.0	5.8	601	0.85	4.6	237
	500	71	3.2	8.1	642	0.52	4.8	230
	750	68	3.3	10.9	589	0.67	4.6	221
	1000	72	3.3	11.8	453	0.57	5.0	228
HIGH (2.6 kg DM/day)	0	73	3.2	8.6	807	0.57	4.3	225
	250	72	3.1	9.7	750	0.68	4.7	216
	500	73	3.4	9.9	732	0.57	4.9	230
	750	72	3.5	13.1	676	0.39	4.8	237
s.e.m.		2.7	0.13	0.96	55.3	0.090	0.20	10.6
Significance								
H		NS	NS	***	*	$P=0.05$	NS	NS
C linear LOW		NS	NS	***	*	NS	NS	NS
C linear HIGH		NS	*	**	NS	NS	NS	NS
H X C		NS	NS	NS	NS	NS	NS	NS

<sup>†</sup> grass DM intake estimated using the n-alkane technique; <sup>‡</sup> BHB = beta hydroxybutyrate; <sup>#</sup>liveweight change from 6 to 1 week pre-lambing

**Conclusions** The results of this study demonstrate the high nutritive value of grass as a feed for twin-bearing ewes in late pregnancy, in view of the satisfactory levels of performance from ewes offered no concentrate supplementation.

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## The effect of steeping on soluble phosphorus levels in wheat, wheatfeed and soyabean meal

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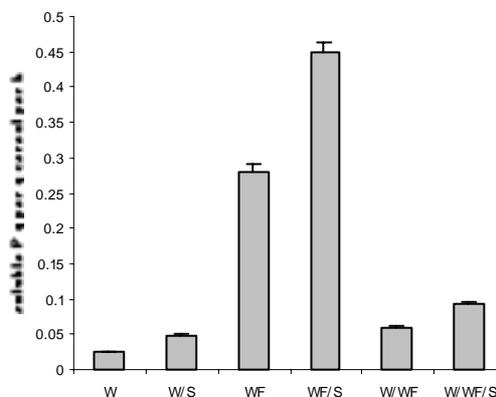
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**Introduction.** Much of the phosphorus in feed ingredients is unavailable to the pig because it is locked up in phytic acid and phytates, which the pig cannot digest. Raw ingredients that have not been heat-treated contain endogenous phytases which can be activated by steeping in water prior to inclusion in liquid feed for pigs. This results in an increase in soluble phosphorus due to the hydrolysis of phytic acid and phytates, and hence an increase in the phosphorus available to the pig. However, with heat-treated ingredients such as soyabean meal (SBM) endogenous enzymes are destroyed during processing. In liquid feed systems steeping combinations of raw materials may have an advantageous affect on the availability of phosphorus as the endogenous phytases of the cereal component of the diet may hydrolyse phytates present in SBM. In wheat the phytate phosphorus and phytase is concentrated in the aleurone layer of the grain (Eeckhout and De Paepe 1994). Therefore, the addition of wheatfeed to liquid feed may increase the amount of endogenous wheat phytase present and hence the amount of soluble P released during steeping. The amount of phosphorus that is available to the pig from these raw ingredients is 30%, 42% and 66% of the total phosphorus for SBM, wheat and wheatfeed respectively (Ewing 1997). The objective of this study was to determine the effect of steeping SBM with wheat, wheatfeed or a combination of the two on the release of soluble phosphorus.

**Materials and Methods.** Wheat and wheatfeed (WF) were steeped alone or in the presence of SBM under optimum conditions for the activity of wheat phytase (pH 5 and 50°C). The proportion of wheat or wheatfeed to SBM in the mixtures was 70:30. A further treatment comprising 10g wheatfeed, 30g SBM and 60g wheat was included to reflect a combination more representative the proportions of these ingredients in a pig diet. Water was added to all treatments to give 300g dry ingredients L<sup>-1</sup>. Three replicates of each treatment were included. The treatments were incubated at 50°C for 7 h under constant agitation and samples taken at appropriate time intervals for the analysis of soluble phosphate. Enzyme activity was stopped by the addition of an equal volume of 10% w/v trichloroacetic acid. Soluble phosphate was determined by suppressed ion chromatography with conductivity detection using a Dionex IonPac<sup>®</sup> AS11-HC analytical column (250 mm x 2 mm *i.d.*) with detection by a Dionex ED50 Electrochemical detector linked to a conductivity cell. The total phosphorus in each raw material was extracted by acid hydrolysis of ashed samples and measured by the above method. The rate of soluble P (g kg<sup>-1</sup> dry ingredients) released per gram of cereal per hour in the mixtures was calculated and compared to that released in by the cereal component alone. Thus the increase in soluble P released due to the presence of SBM in each combination could be calculated.

**Results.** The total phosphorus in wheat, WF and SBM samples was 2.32, 10.5 and 5.33 g kg<sup>-1</sup> respectively. The rate of soluble phosphorus released was significantly increased ( $P < 0.001$ ) in the presence of SBM. This increase represented soluble P released from SBM by phytase activity of the cereal component. The rate of soluble phosphorus release was significantly greater in WF compared with wheat, reflecting the concentration of endogenous phytases in this part of the seed (Figure 1). Although the initial rate of soluble P release differed among treatments, after 7 h steeping there was no significant difference ( $P > 0.05$ ) in the quantity of soluble P released expressed as a percentage of the total P in each mixture. For each treatment the amount of soluble P released was between 93 and 97 % of the total P present in each mixture. This would be equivalent to 3.2, 7.76 and 4.02 g kg<sup>-1</sup> for wheat/SBM, WF/SBM and wheat/WF/SBM mixtures respectively.



**Figure 1.** Rate of soluble P released from wheat (W), wheat + SBM (W/S), wheatfeed (WF), wheatfeed + SBM (WF/S), wheat + wheatfeed (W/WF) and wheat, wheatfeed + SBM (W/WF/S) steeped with water (300g L<sup>-1</sup>) at 50°C

**Conclusions** The endogenous phytase activity of cereals release soluble phosphorus from SBM when these feed components are steeped together. Thus co-steeping ingredients that have phytase activity with those that do not can increase the soluble phosphorus content of liquid feeds. Over 90% of the total phosphorus contained in feed ingredients can be released in this manner. Assuming that all of the soluble P released is available to the pig, this represents a considerable increase in the phosphorus availability of these feed ingredients.

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

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# Performance of grower/finisher pigs fed barley and wheat -based diets containing different levels of a $\beta$ -glucanase and xylanase enzyme combination

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**Introduction** Exogenous enzymes have been used in pig feed to reduce the antinutritional factors in the cereal components of the diets and, as a consequence, improve energy and protein digestibility. The predominant fibre components in barley are soluble  $\beta$ -glucans and insoluble arabinoxylans, both of which are known to have anti-nutritional effects in the pig but through different mechanisms. In wheat both soluble and insoluble arabinoxylans are relevant, for the same reasons (Partridge, 2001). Consequently a product with a combination of  $\beta$ -glucanase and xylanase may be necessary to elicit a performance improvement in pigs fed diets containing both barley and wheat. Furthermore, much work in grower/finisher pigs has been undertaken at single inclusion levels of enzyme product. The object of the current study was to compare the response of growing and finishing pigs fed diets containing barley and wheat supplemented with a  $\beta$ -glucanase and xylanase product included at different levels in the diet.

**Material and Methods** Twenty five male hybrid pigs were individually penned and randomly allocated to one of five treatments from 25 to 110 kg live weight. Treatments were 0, 0.043, 0.085, 0.128, 0.170 g  $\beta$ -glucanase and xylanase dry product/kg (Grindazym<sup>TM</sup> GP15000). The product had minimum guaranteed levels of 15000 BGU/g  $\beta$ -glucanase and 36000 FXU/g xylanase. All treatments were offered *ad libitum* as a two phase grower and finisher feeding regime in which the diets were changed when the pigs reached 65 kg live weight. The main ingredients of the diets (g/kg) were, for the grower and finisher feeds respectively, 350, 340 wheat, 320, 330 barley, 80, 100 wheat feed and 190, 170 hipro soyabean meal and contained 9.31, 9.11 MJ NE/kg and 8.4, 7.4 g digestible lysine /kg. The ingredients were ground (4 mm sieve), mixed and pelleted at below 70 °C. Daily live-weight gain (DLWG) was calculated as the linear slope of the response of live weight (recorded weekly) to time (days) using GENSTAT 5.3 for Windows. The analysis was conducted over the entire duration of the trial. The data were analysed by establishing linear and non-linear (quadratic) contrasts to rate of inclusion of product.

## Results

**Table 1** Effect of a  $\beta$ -glucanase and xylanase enzyme combination on performance of grower and finisher pigs

Treatment	Enzyme product inclusion level (g/kg)					s.e.d.	P values	
	0	0.043	0.085	0.128	0.170		Linear	Quadratic
<i>Grower phase</i>								
Daily live-weight gain (kg)	0.951	0.982	0.994	1.022	1.023	0.052	0.133	NS
Total food intake/pig (kg)	89	88	83	83	86	3.1	0.086	0.148
Food conversion ratio	2.237	2.202	2.087	2.071	2.143	0.079	0.085	0.148
<i>Finisher phase</i>								
Daily live-weight gain (kg)	1.015	1.022	1.003	1.119	1.053	0.057	0.192	NS
Total food intake/pig (kg)	125	124	125	116	124	4.4	NS	NS
Food conversion ratio	2.774	2.755	2.797	2.572	2.766	0.098	NS	NS

Note: NS is  $P > 0.20$ .

Increasing rate of inclusion of  $\beta$ -glucanase and xylanase product resulted in a linear tendency ( $P < 0.10$ ) to reduce food intake (FI) and improve food conversion ratio (FCR) in the grower phase (table 1). Analysis excluding the 0.17 g/kg inclusion level resulted in a linear reduction ( $P = 0.015$ ) in FI and improvement in FCR. Greater DLWG with increasing enzyme supplementation was not significant ( $P < 0.15$ ). The finisher phase showed no significant differences between treatments but, overall, linear trends for FI and FCR were observed ( $P = 0.069$ ) omitting the 0.017 g/kg inclusion level.

**Conclusions** The combination of  $\beta$ -glucanase and xylanase (Grindazym<sup>TM</sup> GP15000) was effective in improving the performance linearly of grower pigs fed diets containing similar levels of wheat and barley (around 300 g of each /kg) up to 0.128 g/kg, but reduced at the 0.17 g/kg inclusion level. Due to the apparently reduced effects at the highest enzyme level tested, further work on the effects of variable levels of enzymes in-feed should be undertaken to clarify optimal inclusion levels. In this trial, at 0.128 g/kg, FCR was improved by 7% and DLWG increased by 8% compared with the control treatment in the grower phase. It is noteworthy that similar proportional increases were observed between the same treatments in the finisher phase, but no linear or quadratic trends were observed. It may be hypothesised that the use of fibre-digesting enzymes released nutrients, which would otherwise have been bound in the fibre components of the grain. Pigs were unable to achieve optimum grower performance without the improved diet nutritional value offered by the enzymes, possibly due to constraints in appetite often observed at this age. However, performance may not have been so limited in the finisher phase due to the relatively high feed intake of *ad libitum* fed individually-housed pigs that permitted sufficient nutrient intake. This could be tested in group-housed pigs in commercial conditions in which food intake is usually lower.

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## The use of phytase in finishing pig diets

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**Introduction** Phosphorus (P) is an essential mineral for pigs and deficiency can cause rickets, osteomalacia or osteoporosis and has been associated with poor fertility and production performance. Traditionally P has been supplied in the inorganic form as the organic form found in cereal grain (phytic acid) is unavailable to pigs as they lack endogenous phytase. Dietary phytases have been shown to play a major role in pig diets, releasing P from phytic acid thus decreasing the need for supplementary P and reducing P excretion (Harper *et al* 1997). Phytase supplementation has also been reported to improve digestibility of nitrogen, calcium, magnesium, zinc and copper (Jongbloed *et al* 1993). Some researchers (e.g. Jongbloed and Kemme 1990) suggest that diet processing may reduce enzyme activity and therefore liquid enzyme products have been developed which can be applied after processing. The aims of this study were to examine the effectiveness of several phytase products (including dry vs. liquid) in improving overall digestibility and reducing excretion of P and other nutrients.

**Materials and Methods** A finishing diet formulated to contain g/kg; barley, 565, wheat, 100, soyabean meal, 230, soya oil, 30, binder 50 and minerals and vitamins 25, was supplemented with a range of dry (n=5) or liquid (n=4) phytase products (*Aspergillus Niger*). The inclusion level for the phytase products was between 0.1 and 0.3g/kg (according to manufacturers recommendations). There were a total of ten treatments (T1-5 + dry phytase, T6-9 + liquid phytase and T10 no phytase, control) fed to 80 boars (45kg) housed in metabolism crates in 8 consecutive replicates. Each replicate comprised a 7 day pre-feed and a 7 day faecal collection. Samples of the diets, faeces and urine were collected and analysed to determine digestibility and mineral retention / excretion coefficients. The results were analysed by ANOVA using Genstat 5.

**Results** Supplementation with the phytase products (T1-9) improved digestibility of P and reduced P excretion (P <0.001 and <0.01 respectively). Neither digestibility nor excretion of nitrogen (N) were affected by phytase supplementation (Table 1). Both the dry (55.4%) and liquid (55.5%) forms of phytase resulted in an improvement (P <0.001) in P digestibility when compared to the control treatment (46.1%). However, there was no significant difference between the response achieved by either the dry or liquid forms.

**Table 1.** The effect of a range of dry and liquid phytase products (T1-9) on the digestibility, retention and excretion of P and N relative to control (T10)

	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	sem	Prob
Digestibility of P (%)	59.1	53.5	54.5	56.5	53.7	57.8	55.4	54.1	54.7	46.1	1.57	<0.001
P retained (g/d)	4.47	3.87	3.97	4.21	3.74	4.14	4.16	4.07	4.05	3.30	0.147	<0.001
P excreted (g/d)	3.38	3.63	3.53	3.37	3.58	3.27	3.54	3.69	3.63	4.20	0.144	<0.01
Digestibility of N (%)	83.0	81.2	82.2	80.8	82.9	83.2	81.4	82.0	81.5	81.5	0.75	NS
N retained (g/d)	35.9	33.1	33.6	34.6	32.2	34.1	33.5	34.4	34.0	34.2	0.99	NS
N excreted (g/d)	20.6	20.9	20.5	20.1	20.4	19.3	21.9	21.5	21.3	19.8	0.70	NS

**Conclusion** All the phytase products were effective in improving digestibility of P and reducing P excretion, which is in line with the results of Harper *et al* (1997). Phytase had no effect on digestibility or excretion of N, which is in contrast to the report by Jongbloed *et al* (1993). Both liquid and dry phytase products were equally effective in improving P digestibility.

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## Application and comparison of two models of phosphorus flows in growing pigs

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**Introduction** Studies of phosphorus (P) metabolism often involves balance trials and use of isotopes. When combined with mathematical modelling, calculation of flows between several pools becomes a possibility. A key objective of the study on pigs employing isotope and balance techniques reported herein was to apply and compare two models of P metabolism for resolving data generated by these techniques.

**Materials and Methods** Ten crossbred Landrace x Large White castrated males at 19.7 kg ± 0.5 live weight were allocated to five groups and fed diets based on corn and soybean meal and consisting of different total P levels: 0.30, 0.40, 0.51, 0.65 and 0.73 g/100 g DM. The calcium intake was maintained at 0.60 g/100 g DM in all five groups. Following a single dose injection of <sup>32</sup>P into the jugular vein, blood samples, faeces and urine were collected at 24 h intervals for 7 days. Samples of tissue (liver, heart, kidney and *m. Longissimus dorsi*) and bone (13<sup>th</sup> and 14<sup>th</sup> rib) were also collected and total P and radioactivity in all samples were measured. Two mathematical models were applied to analyse data. The model of Vitti *et al.* (2000) describes P circulation between four pools: gut, blood, bone and smooth tissues. The model of Fernández, (1995) describes P circulation between P pools in gut, blood and bone.

**Results** Phosphorus flows for each intake level calculated using the model of Vitti *et al.* (2000) are shown in Table 1. Flow increased in both directions between gut and blood with increasing intake ( $P < 0.05$ ). Phosphorus bone absorption and re-absorption were not affected by treatment. However, pigs on low P intakes transferred a relatively greater amount of absorbed P to the bone (6.10, 4.10, 2.44, 2.55 and 1.82 g P/day) and from bone (4.99, 2.63, 2.05, 1.77 and 0.96 g P/day) than pigs on high P intakes. The differences were not statistically significant. The flows from blood to soft tissue were higher in the pigs on the medium P intake than the other pigs ( $P < 0.01$ ). A similar, and non-significant, pattern was seen in the opposite direction. Flows calculated using the model of Fernández (1995) showed that the forward and backward P flows between gut and blood increased with increasing intake (Table 2). The same amount of P was absorbed into bone for all five intakes, while re-absorption from bone decreased with increasing P intake.

The two models agreed on absorption rates from gut to blood, while the flow from blood to gut increased more slowly with the Vitti model than when using the Fernández model. Phosphorus incorporation into bone appeared to increase a little faster in the Vitti model. The Fernández model gave a decreasing reabsorption from bone, which in the Vitti model was uninfluenced by P intake. Although flows to and from bone differed, the models gave an identical increasing P net retention in bone with increasing intake.

**Conclusions** Increasing P intake increased flow bi-directionally between gut and blood as well as net retention in bone. The two models compared were similar in principal, although their aims and therefore calculations differed.

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**Table 1.** Flows calculated using the model of Vitti *et al.* (2000) for different P intake levels in pigs (treatment means)

Item	Intake, g P/ day					SEM
	1.88	2.92	3.83	4.55	5.63	
Model output (g P/day)						
Gut to blood	0.89 <sup>x</sup>	1.75 <sup>y</sup>	3.36 <sup>z</sup>	3.22 <sup>z</sup>	3.88 <sup>z</sup>	0.38
Blood to gut	0.25 <sup>x</sup>	0.35 <sup>x</sup>	0.87 <sup>y</sup>	0.55 <sup>xy</sup>	0.61 <sup>y</sup>	0.08
Blood to bone	5.23	6.74	8.14	8.24	7.12	0.51
Bone to blood	4.23	4.18	6.79	5.71	3.79	0.54
Blood to tissues	1.17 <sup>x</sup>	1.49 <sup>x</sup>	3.42 <sup>y</sup>	1.67 <sup>x</sup>	0.95 <sup>x</sup>	0.30
Tissues to blood	1.58	2.65	2.45	1.84	1.53	0.18

<sup>x,y,z</sup> Within a row, means with different superscripts differ ( $P < 0.05$ ).

**Table 2.** Flows calculated using the model of Fernández (1995) for different P intake levels in pigs (treatment means)

Item	Intake, g P/ day					SEM
	1.88	2.92	3.83	4.55	5.63	
Model output (g P/day)						
Gut to blood	0.43	2.47	3.03	4.44	5.08	0.57
Blood to gut	-0.21	1.07	0.53	1.77	1.81	0.30
Blood to bone	2.73	3.05	3.59	3.20	2.70	0.17
Bone to blood	2.14	1.65	1.27	0.86	-0.05	0.29

# The effect on litter performance at birth of feeding gilts fermented liquid feed (FLF), non-fermented liquid feed (NFLF) or dry feed (DF) for 14 days pre-farrowing

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**Introduction** Feeding and management during gestation focuses on preparing the sow for parturition and lactation. Despite improvements in husbandry in recent years, mortality remains around 10% and may be increasing (Herpin *et al.*, (1993). Cole, (1990) found that foetal growth rate in the last trimester of pregnancy increases dramatically compared with early and mid-gestation and Cromwell *et al.*, (1989) reported that a 75% increase in feed intake during the last 23 days of gestation increased reproductive performance and also increased birth weight of piglets (1.48 vs. 1.44,  $P < 0.003$ ). Results from previous studies have also shown that an increase in feed intake from 2.3 kg to 3.9 kg per day can reduce sow backfat loss during the reproductive cycle and increases reproductive life (Miller *et al.*, 2000). The study reported here investigated the influence of three different diet forms fed to gestating gilts for 14 days pre-farrowing, on litter characteristics at birth.

**Materials and methods** A study was conducted according to a randomised block design with 2 replicates. Eighteen gilts (Large White x Landrace) were randomly allocated to one of the three dietary treatments namely: fermented liquid feed (FLF), non-fermented liquid feed (NFLF) and dry feed (DF) in pelleted form. The liquid diets were made by mixing the DF offered for the third treatment at 2:1 water to feed ratio. An amount of 3 kg was measured daily for the preparation of next day's feeding. *Lactobacillus salivarius* of pig origin was used as a starter culture for FLF. After 24-hours sanitization 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  $\text{ml}^{-1}$  liquid feed. The inoculated feed was fermented for 36 hours at 30°C. The diets had a specification designed to meet the nutritional requirements of the gestating and lactating gilts and to maintain normal health and vigour. Feeding took place twice a day for a period of 2 weeks before farrowing date, and for 3 weeks after farrowing according to MLC's Stotfold Feeding Scale for lactating sows. In gestation the gilts were loose-housed in-groups of 6 in straw-bedded pens, which were provided with individual feeders. Water was provided *ad libitum*. Gilts were moved to farrowing crates 4 days prior to their anticipated farrowing date. Each of the gilts was individually penned in a farrowing crate with water provided *ad libitum*. All piglets were weighed at birth. The dry matter of fermented and non-fermented feed was determined daily by oven drying at 103°C for 3-4 days (Method: ISO 6469/NEN 3332). The data were analysed using a GLM-ANOVA.

## Results

**Table 1** Effect of type and amount of feed on litter performance at birth.

Measurements (average)	FLF (1)	NFLF (2)	DF (3)	SED (1-2)	SED (1-3)	SED (2-3)
Litter size	11.37	11.27	10.15	0.319	0.351 **	0.341
Piglets alive per litter	11.13	9.72	9.79	0.742	0.699	0.343
Birth-weight (kg) (BW)	1.787	1.401	1.332	0.122 **	0.118 ***	0.056
Feed Intake DM (kg) during 2 weeks period	33.98	36.90	36.61	0.100 ****	0.110 ****	0.107 *

\* $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ , \*\*\*\*  $P < 0.0001$

Piglets from gilts fed FLF were 300 and 450g heavier than piglets from the gilts fed NFLF ( $P < 0.01$ ) and DF fed gilts ( $P < 0.001$ ) (Table 1). The number of piglets remained alive was numerically higher for sows fed on FLF.

**Conclusions** The results from this study support the view that the provision of FLF to gilts at 14 days before farrowing, can improve the BW of piglets compared with the birth weight of piglets from gilts fed NFLF and DF, even though the sows on FLF consumed less DM ( $P < 0.0001$ ) and had significantly larger litters ( $P < 0.01$ ) than gilts fed DF.

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# Fermented liquid feed can reduce *E. coli* bloom at farrowing and prevent constipation problems during lactation

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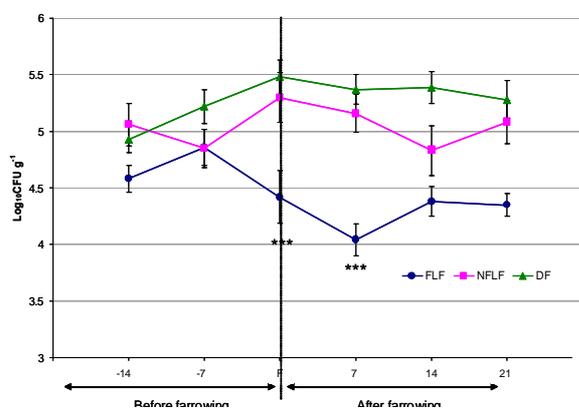
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**Introduction.** Gastrointestinal infections associated with *E. coli* represent a serious problem for neonatal pigs. These bacteria are present in the sow's intestine in large numbers but increase dramatically just prior to farrowing due to stress occasioned by movement and parturition (Maclean and Thomas, 1974). Consequently, just 24 hours after farrowing, *E. coli* are found in high numbers (over  $10^8$ /g) in the faeces of piglets. However, at this stage of its life the piglet is not equipped to deal with such a large microbial load and unless immunological assistance is provided, they have very little chance of survival. In pigs, all immunological assistance at birth is concentrated in the mother's colostrum. Thus elimination, or at least minimizing, all the factors which negatively affect the sow's ability to produce sufficient amount of milk becomes essential challenge of each efficient swine production. The main aim of this study was to investigate the potential of fermented liquid feed (FLF) to control the pathogen load within the piglet's environment by reducing the rapid *E. coli* multiplication in sows associated with farrowing. The possible laxative effect of FLF, in order to prevent constipation and the problems it causes during farrowing, was also examined.

**Materials and methods.** A study was conducted according to a randomised block design, with two replicates. Eighteen gilts (Large White x Landrace) were randomly allocated to one of the three dietary treatments namely: fermented liquid feed (FLF), non-fermented liquid feed (NFLF) and dry feed (DF) in pelleted form. The liquid diets were made by mixing the DF offered for the third treatment at 2:1 water to feed ratio. *Lactobacillus salivarius* of pig origin was used as a starter culture for FLF. After 24-hours sanitization 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  $\text{ml}^{-1}$  liquid feed. The inoculated feed was fermented for 36 hours at 30°C. Feeding took place twice a day for a period of 2 weeks before farrowing date, and for 3 weeks after farrowing according to MLC's Stotfold Feeding Scale for lactating sows (MLC, 1999). Fresh faecal samples were collected from the rectum of each sow weekly during the experimental period. Dry matter concentration of each faecal sample was determined by oven drying at 103°C for 3 days. *E. coli* were analysed in each faecal sample by standard methods. The bacteria count per gram of faeces was log transformed, tabulated, and statistically analysed by ANOVA. Significant differences between treatment means were compared by Tukey's HSD test. Statistical analyses were undertaken using Minitab v 13.31 (Minitab Inc., Pennsylvania, USA, 2000).

**Results.** In sows fed DF and NFLF the *E. coli* population increases considerably during the two weeks pre-partum and remained high throughout lactation (Figure 1). In contrast, the *E. coli* population declined for three weeks after the commencement of feeding FLF. The numbers of *E. coli* in faeces of sows fed FLF were significantly lower at birth and at 7 days post-partum and remained lower throughout lactation. At farrowing, the dry matter concentration of faeces from both FLF and NFLF fed sows were significantly lower than that of DF fed sows (Table 1). In addition sows fed FLF, but not sows fed NFLF, had faeces with lower DM content for 14 days postpartum.

**Figure 1** Numbers of *E. coli* ( $\log_{10}$  cfu  $\text{g}^{-1}$ )  $\pm$  SEM in the gilt's faeces 14, 7 days before farrowing, at farrowing and 7, 14 and 21 days postfarrowing.



**Table 1** Dry matter content (g/kg) of faeces of gilts fed FLF, NFLF, and DF during period 14, 7 days before farrowing, at farrowing and 7, 14 days postfarrowing.

	FLF	NFLF	DF	P
Day 14 BF	24.71±0.4	26.31±0.6	25.38±0.5	ns
Day 7 BF	27.04±0.7	28.34±0.5	26.37±0.6	ns
Farrowing	27.93±0.2 <sup>a</sup>	27.81±0.4 <sup>a</sup>	30.40±0.4 <sup>b</sup>	***
Day 7 AF	23.33±0.7 <sup>a</sup>	25.91±0.8 <sup>b</sup>	27.99±0.8 <sup>b</sup>	***
Day 14 AF	22.47±0.6 <sup>a</sup>	26.64±0.6 <sup>b</sup>	26.26±0.7 <sup>b</sup>	***

BF- before farrowing; AF-after farrowing; \*\*\*P<0.001; <sup>a,b</sup> within the rows, means with the same superscript are not significantly different. Data are expressed as a mean  $\pm$  SEM

**Conclusions** These results demonstrate that the *E. coli* challenge to the newborn piglet can be reduced by feeding sows fermented liquid feed. Furthermore, FLF has a laxative effect which may reduce the incidence of constipation in sows and the associated problems. Together these changes could result in improved sow and litter performances and profitability.

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# Effect on average daily feed intake during lactation and piglet growth during the first 2 weeks of life of feeding sows fermented liquid feed, non-fermented liquid feed or dry feed

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**Introduction** Achieving adequate feed intakes by sows, and particularly gilts, in lactation can often be a problem under intensive farrowing-house conditions. It has been shown that sow feed intake during lactation can significantly affect subsequent reproductive efficiency such as remating interval, pregnancy rate and embryo survival (Einarsson and Rojkittikhun, 1993). There are many nutritional, environmental and physiological factors that influence the sow's appetite during lactation. The physical form of the diet (wet or dry) has also been shown to affect feed intake as sows ate 12 % more feed given as a wet mash compare with dry form (O'Grady and Lynch, 1978; Genest and Dallaire 1995). The growth of the piglets during the first 2 weeks of lactation depends entirely on the milk supply of the mother. As this has been shown to be influenced by the level of feed intake (Koketsu *et al.*, 1996), factors controlling feed intake during lactation should determine the rate and efficiency of piglet growth. The aim of this study was to investigate the effect of three different forms of diet on lactation feed intake of gilts and growth performance of their piglets.

**Materials and methods** A study was conducted according to a randomised block design with 2 replicates. Eighteen 35 weeks old gilts (Large White x Landrace) were randomly allocated to one of the three dietary treatments namely: fermented liquid feed (FLF), non-fermented liquid feed (NFLF) and dry feed (DF) in pelleted form (BOCM Pauls, 14MJ/kg of digestible energy). The liquid diets were made by mixing the DF offered for the third treatment at 2:1 water to feed ratio. Each day 3 kg was prepared for feeding the next day. Feeding took place twice a day for a period of 2 weeks before farrowing date, and for 3 weeks after farrowing according to MLC's Stotfold Feeding Scale for lactating sows. In gestation, the gilts were loose housed in groups of 6 in straw bedded pens, which were provided with individual feeders. Water was provided *ad libitum*. Gilts were moved to farrowing crates 4 days prior to their anticipated farrowing date. All piglets were weighed at birth, after first and second week of suckling. The dry matter of FLF and NFLF was determined daily by oven drying at 103°C for 3-4 days (Method: ISO 6469/NEN 3332). The data were analysed using a GLM-ANOVA.

**Results** Average daily feed intakes of sows fed liquid diets were significantly higher (15-20%) than those of DF sows during the second and third week of lactation (Table 2). However, because of the small number of replicates there was no significant relationship with piglet growth (Table 1).

**Table 1.** Average piglet weight (kg) after first and second week of suckling.

	FLF (1)	NFLF (2)	DF (3)	sed (1-2)	sed (1-3)	sed (2-3)
Week 1	2.71	2.89	2.72	0.10	0.10	0.12
Week 2	4.00	4.39	4.39	0.14	0.16	0.18

\*P<0.05

**Table 2** Average daily feed intake ADFI (DM kg/day) of gilts fed FLF, NFLF, and DF during 3-week lactation period.

	FLF (1)	NFLF (2)	DF (3)	sed (1-2)	sed (1-3)	sed (2-3)
Week 1	3.2	3.5	3.0	0.20	0.20	0.21
Week 2	5.6	6.0	4.8	0.21	0.22	0.23
Week 3	7.1	7.4	6.0	0.21	0.21	0.23

\*\*\*P<0.001, \*\*\*\*P<0.0001;

**Conclusions** These results are in agreement with some previous studies which also concluded that energy intake during lactation have no or only very little effect on piglet growth (Genest and Dallaire 1995). Even on wet diets, where the sow ate 12% more per day than those on dry feed, the difference in nutrient uptake were reflected in sow weight change and not in litter growth (O'Grady and Lynch, 1978). However, the demands of lactation are considerable and if not met by the diet they must be met from the maternal reserves. This higher ADFI of FLF and NFLF-fed gilts may result in better subsequent reproductive efficiency as it was shown in recent study by Eissen *et al.*, 2000. Higher feed intake during lactation tends to reduce tissue loss by the sow and reduces the probability of a prolonged weaning-to-oestrus interval. In addition, sows with low feed intakes in lactation tend to produce fewer piglets in the subsequent litter (Hughes, 1993).

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# Effect of dietary salt (NaCl) level on the growth performance of liquid fed growing-finishing pigs.

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**Introduction.** Salt (NaCl) is an essential mineral and its rate of inclusion in diets has been implicated in the development and expression of biting behaviour. Falkowski *et al.*, (1998) found that withholding salt from diets reduced the feed intake and feed conversion ratio of weaned pigs and significantly decreased growth rate. Studies reported by Fraser (1987), suggested that heightened appetite for salt could make pigs particularly attracted to pen mates with injured tails. More recently Tsourgiannis, *et al.*, (2002) reported that 1.5% salt inclusion levels can significantly reduce the incidence of tail-biting, providing that there is unrestricted access to fresh water. This study investigated the effect on growth performance of pigs fed liquid diets containing high and low dietary salt levels for growing/finishing pigs.

**Materials and methods.** The experiment was designed to examine the effect that dietary salt inclusion level had on feed and water intake and biological performance of liquid fed growing-fattening pigs. The experiment was conducted using a cross-over design with two replicates, two periods and two treatments (2x2x2). Each treatment consisted of 16 pigs (8 male and 8 female) averaging 38.8±1.1 kg, four pens (two replicates of each dietary treatment) each containing 8 pigs (four male and four female). Each replicate consisted of two treatments: a high quality commercial diet with 0.25% added salt, and second experimental group fed the same basal diet but containing 1% added salt. Two randomly chosen pens of pigs were treated using one of the two diets for one period (two weeks) and then changed to the second treatment for the rest of the experiment. A commercial diet was formulated with no added salt. The pigs were offered the experimental diets at 2.5 parts water to one part of food *ad lib*. The salt was dissolved in the water before adding the basal diet to ensure even distribution. The diets differed only in their salt content. Feed refusals were collected daily, and samples dried in order to calculate the DM of residual food. Freshly mixed food was added twice a day. Feed was provided in all feeding troughs (8 troughs) of each pen, to ensure that all pigs could feed simultaneously if they wished. Fresh water was supplied through a drinker in each pen and water consumption was recorded daily per pen using a water meter (Kent PSM-L). The pigs were weighed on the first and last day of the trial and every 7 days, for four weeks (final live-weight 68±1.1 kg). The data were analysed using a GLM-ANOVA.

## Results.

**Table 1** Summary of recordings during the period of the experiment.

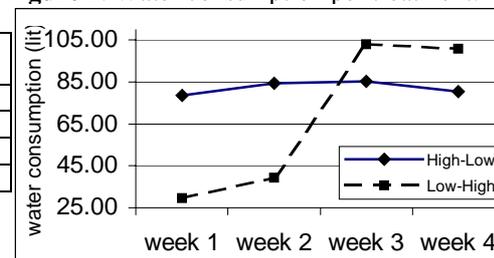
Period	Weight Gain/period/pig (kg)			Feed Intake DM/week/pig (kg)			Water Consumption/pig/week (lit)		
	High	Low	SED	High	Low	SED	High	Low	SED
0-2 weeks	6.77 <sup>a</sup>	6.73 <sup>b</sup>	0.37	12.14 <sup>a</sup>	12.36 <sup>b</sup>	0.80	11.96 <sup>a</sup>	4.94 <sup>b</sup>	0.649*
2-4 weeks	6.01 <sup>b</sup>	6.03 <sup>a</sup>	0.31	13.59 <sup>b</sup>	14.33 <sup>a</sup>	0.89	14.55 <sup>b</sup>	11.8 <sup>a</sup>	0.863*
Over All	6.39	6.37	0.24	12.87	13.45	0.49	13.26	8.39	0.492*

\* Statistically different (P<0.001)

The values in the table represent the average performance of pigs in each pen.

Values with the same exponential letter represent pigs from the same group of pens.

**Figure 1.** Water consumption per treatment.



There were no significant differences between the treatments for feed intake and weight gain. Pigs fed on 0.25% NaCl diet consumed 58.7% less water (Figure 1) than pigs fed the diet containing 1% salt during the first period. Pigs fed the 1% salt diet, continued to consume high levels of water even when they changed to the 0.25% NaCl diet for the rest of the experimental period. Water intakes continued to be significantly greater (P<0.001) than those of pigs fed on low salt inclusion diet.

## Conclusions.

Additional salt is often added to diets for grow-finish pigs to reduce the incidence of tail biting. The results obtained in this study indicate that increasing the salt concentration from 0.25 to 1.0% does not adversely affect the dry matter feed intake and growth performance of pigs. However, such an increase in salt content did increase water consumption by 58.7%. This would have important implications for effluent production.

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# Modelling the effects of the thermal environment and dietary composition on pig performance

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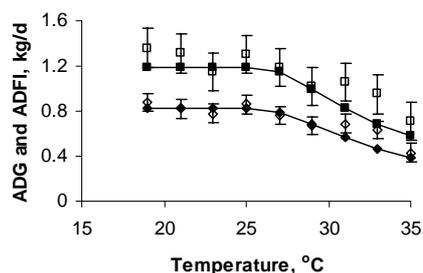
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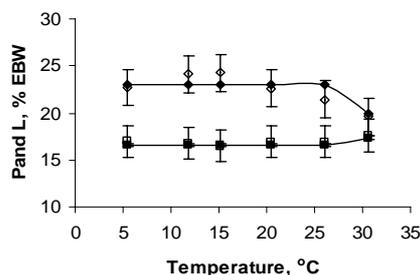
**Introduction** Simulation models allow the effects of a range of environmental and other variables on animal performance to be considered simultaneously in a way that cannot be done by direct experimentation. Consequently, limiting factors within a system can be identified, the effects of genetic selection predicted and areas needing further research highlighted. The aim here is to develop, test and evaluate a deterministic dynamic model that predicts over time the effect of genotype, the nutritional and thermal environments, including any interactions, on the voluntary feed intake (VFI), growth and body composition of pigs.

**Materials and Methods** From the daily potential for protein gain as determined by pig genotype and current state, the potential gains of the other body chemical components, including 'desired' lipid gain, are calculated. Unconstrained VFI, predicted from the current state of the pig and the composition of the feed, is that needed to fulfil potential growth. The model allows compensatory lipid gain. Feed composition is described in terms of digestible energy content, ideal digestible crude protein content and bulkiness. Both energy and protein may be limiting and feed bulkiness may constrain intake depending on the animal's capacity for bulk. The thermal environment, as described by the ambient temperature, wind speed, floor type and humidity, sets the maximum ( $HL_{max}$ ) and minimum ( $HL_{min}$ ) heat the pig is able to lose. A comparison with heat production (HP) determines whether the environment is hot ( $HP > HL_{max}$ ), cold ( $HP < HL_{min}$ ) or thermoneutral ( $HL_{min} < HP < HL_{max}$ ). If hot a constraint on VFI is imposed, while if cold an extra thermal demand is placed upon the pig. In thermoneutral conditions no further action is taken. Daily gains of the chemical components are calculated by partitioning energy and ideal protein between protein and lipid retention according only to the energy to protein ratio of the feed. Several well-described data sets from the literature, all meeting the necessary requirement of feeding the pigs *ad libitum* and reporting reasonable information on the trial conditions, used as model inputs, were used to assess the appropriateness and 'value' of the model over a wide range of treatments.

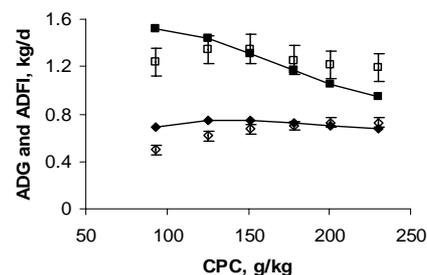
**Results** The model predicts with a reasonable degree of accuracy, the effects of differing temperature (Figures 1 and 2) and crude protein content (CPC) (Figure 3), including any interactions, on the average daily intake (ADFI), daily gain (ADG) (Figures 1 and 3) and body composition (Figure 2) of pigs.



**Figure 1** Observed ( $\pm$  sd) ( $\square$ ,  $\diamond$ ) and predicted ( $\blacksquare$ ,  $\blacklozenge$ ) effects of temperature on the ADFI ( $\square$ ,  $\blacksquare$ ) and ADG ( $\diamond$ ,  $\blacklozenge$ ) for the pigs of Collin et al. (2001)



**Figure 2** Observed ( $\pm$  sd) ( $\square$ ,  $\diamond$ ) and predicted ( $\blacksquare$ ,  $\blacklozenge$ ) effects of temperature on final body protein ( $\square$ ,  $\blacksquare$ ) and lipid content ( $\diamond$ ,  $\blacklozenge$ ) for the pigs of Nienaber et al. (1987)



**Figure 3** Observed ( $\pm$  sd) ( $\square$ ,  $\diamond$ ) and predicted ( $\blacksquare$ ,  $\blacklozenge$ ) effects of CPC on ADFI ( $\square$ ,  $\blacksquare$ ) and ADG ( $\diamond$ ,  $\blacklozenge$ ) for the pigs of Ferguson and Gous (1997)

**Conclusion** The direction of response of pig growth and intake observed in the experiments where temperature and CPC varied, was correctly predicted by the model. This should be regarded as giving support to the model's adequacy rather than testing its strict predictive accuracy. Where model predictions differed quantitatively from those observed, it was thought to be due to a greater sensitivity of the model to temperature, probably due to the omission of long-term adaptation and acclimatisation, or an incorrect estimation of the wetness of the pig's skin. This approach builds on other models by predicting the feed intake and growth of growing pigs in differing dietary and thermal environments, whilst maintaining simplicity and flexibility enabling it to adapt to continued changes in genetics and production systems.

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

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# Supplementation with rumen inert fat of late pregnant dairy cows modifies the relationship between body condition and plasma leptin concentration

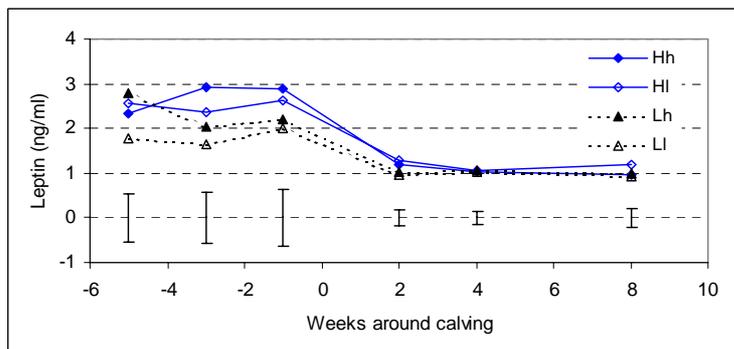
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**Introduction** The association among feeding, body fat reserves, plasma leptin concentration and intake has been indicated in many reports, however the characteristics of these associations in late pregnant ruminants is not yet completely clear. As part of a larger experiment concerned with nutrition during the dry period (DP), a study was undertaken to identify the relationship between precalving fat and protein supplementation with plasma leptin concentration and the association of leptin with fat reserves and dry matter intake (DMI) during the periparturient period. Further results associated with this experiment were reported elsewhere (Jaurena *et al.*, 2001a,b; Jaurena *et al.*, 2003).

**Material and methods** Twenty four mature (parity > 2) Holstein-Friesian dairy cows were allocated to one of four treatments in a factorial arrangement of rumen inert fat (F, Megalac<sup>TM</sup>, a Ca soap made from palm oil) and protein (P), *i.e.* low-P, low F (Ll): first cut ryegrass silage only; (Lh): the same silage with 10 % rumen inert fat mixed on a dry matter (DM) basis); high-P, low-F (Hl): silage with 5 % prairie meal; high P, high-F (Hh): silage with 5 % prairie meal and 10 % Megalac. All the diets were individually offered *ad libitum* for the last 6 weeks of the DP. After calving all cows received the same ration: ryegrass silage plus 8 kg/day of a commercial dairy concentrate (13.5 MJ metabolisable energy and 240 g crude protein per kg DM). Dry matter intake was recorded daily from 6 weeks before calving. Animal back fat depth (bckF, measured with an ultrasound scanner at the 5<sup>th</sup> lumbar process) and BCS (0-5 scale) were measured weekly between 5 weeks before calving and 8 weeks of lactation. Blood samples were taken at weeks -5, -3, -1, +2, +4 and +8 relative to calving from the jugular vein or coccygeal vessels into lithium heparinised evacuated tubes. Results were analysed by analyses of variance and covariance, with a factorial treatment structure of diet F×P, and by correlation analysis.



**Figure 1** Plasma leptin concentrations around calving in cows fed with Ll, Lh, Hl and Hh diets during the dry period. Vertical bars, pooled standard deviation.

animals not receiving fat supplement ( $r = 0.82$ ,  $P = 0.015$ ), but no significant association was found within the group of cows receiving fat-supplement at week -5 and at weeks -3 and -1 of calving. No significant associations between bckF at weeks -5, -3 and -1 with the respective plasma leptin concentrations were found ( $P > 0.15$ ). Plasma leptin concentration and DMI showed significant ( $P < 0.05$ ) correlation coefficients between leptin at week -5 and DMI at weeks 4 and 8 of lactation ( $r = 0.45$  and  $r = 0.50$  respectively) and plasma leptin concentration at week 2 postpartum with DMI at week 8 of lactation ( $r = 0.56$ ).

**Conclusions** It is concluded that the positive association between BCS and plasma leptin concentration is changed by feeding rumen inert fat during the late DP. It is also concluded that concentration of leptin in plasma is affected by dietary composition. The lack of association between bckF and plasma leptin concentration might have been due to the generally thin state of the animals and narrow range of bckF variation.

**Acknowledgements** This work was funded by the Department for the Environment, food and Rural Affairs.

G. Jaurena is grateful for scholarships provided by the British Council and Fundación Antorchas (Argentina). We are grateful to Prof. Bob Webb (University of Nottingham) for leptin analysis.

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## Plasma metabolite and hormone responses to rumen inert fat and protein supplies during the dry period in dairy cows

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**Introduction** Nutritional regime during the dry period (DP) has been shown to affect lactational performance in dairy cows. These responses could be mediated by a variety of mechanisms (*e.g.* increased labile body proteins, function of the mammary gland or the liver, or modifying endocrine status), which are likely to exert an overlapping function. This study analysed the effect of protein (P) and/or fat (F) supplementation in the DP on plasma metabolite and hormone concentrations, as a complement to studies on body composition (Jaurena *et al.* 2001b) and subsequent milk production (Jaurena *et al.*, 2001a) performance. Other results also associated with this work are presented in Jaurena *et al.* (2003).

**Material and methods** Forty Holstein-Friesian dairy cows were allocated to one of four feeding treatments, *i.e.* low-P, low F (Ll): first cut ryegrass silage only; (Lh): the same silage with 10 % rumen inert fat (Megalac<sup>TM</sup>, a Ca soap made from palm oil), mixed on a dry matter (DM) basis; high-P, low-F (Hl): silage with 5 % prairie meal (PM); high P, high-F (Hh): silage with 5 % PM and 10 % Megalac. All the diets were individually offered *ad libitum* for the last 6 weeks of the DP. After calving all cows received the same ration: ryegrass silage plus 8 kg/day of a commercial dairy concentrate (13.5 MJ metabolisable energy and 240 g crude protein (CP) per kg DM). Blood samples were collected from the jugular or coccygeal veins weekly from six weeks before calving and at weeks 1, 2, 4, 8, 12, 16 and 20 of lactation. Results were analysed by ANOVA as a factorial (2×2×2) arrangement of maturity (Young, Y, n = 4; Mature, M, n = 6), P (High/Low), and F (high/low) in a complete randomised design.

**Table 1.** Precalving plasma concentration of metabolites and hormones affected by fat or protein supplementation in the dry period.

Variable <sup>†</sup>	Fat		Protein		P <sup>‡</sup>
	low	high	Low	High	
NEFA (mmol/l)	0.32	0.56	0.39	0.49	F*,P*
BHBA (mmol/l)	0.48	0.39	---	---	F*
Urea (mmol/l)	8.7	7.9	7.8	8.9	F*,P*
Progesterone (ng/ml)	6.1	6.7	---	---	F*
Growth hormone (ng/ml)	---	---	21	16	P*

<sup>†</sup> NEFA, non-esterified fatty acids; BHBA,  $\beta$ -hydroxybutyrate.

<sup>‡</sup> Significant factors; F, fat; P, protein; \*,  $P < 0.05$ .

presented in Table 1. Peak concentration of prolactin in plasma of Y cows differed between Lh (16 ng/ml) and Ll (86 ng/ml) treatments ( $P < 0.05$ ). Mean plasma prolactin concentration (3 last weeks of gestation) also showed a significant interaction (for M cows, low F = 11, high F = 18 ng/ml; and for Y cows low F = 17 and high F = 10 ng/ml;  $P_{M \times F} = 0.005$ ). Supplementation with P decreased the GH peak in Y animals (43 to 29 ng/ml;  $P_{M \times P} = 0.011$ ). During the period of 4 to 8 weeks of lactation there was a M×P×F interaction for plasma concentrations of TP (Y-Ll = 67; Y-Lh = 78 g/l;  $P < 0.05$ ), Glo (Y-Ll = 29; Y-Lh = 40;  $P < 0.10$ ) and urea (M-Lh = 10.2; M-Hh = 8.1 mmol/l;  $P < 0.01$ ). Fat supplementation in the DP increased plasma NEFA concentration (low F = 0.31; high F = 0.45 mmol/l;  $P < 0.039$ ). No differences were observed for Alb, glucose and BHBA. In the period of 12 to 20 weeks of lactation an M×P×F interaction for plasma concentrations of urea (M-Lh = 11.8; M-Hh = 8.7 mmol/l;  $P < 0.012$ ) was observed and plasma glucose concentration was reduced from 5.2 to 4.9 mmol/l by previous supplementation with either F or P ( $P < 0.05$ ).

**Conclusion** Fat supplementation seems to have improved the energy status of the animals during the DP, as indicated by BHBA, but as plasma NEFA concentrations during the DP were also increased, it could have increased hepatic uptake of NEFA. Plasma concentrations of GH and Prg were influenced by P and F supply respectively during the DP. Maturity increased GH and interacted with diet to determine plasma prolactin concentrations. Residual effects of DP F or P supplementation were observed on NEFA, TP, Glo, Urea and glucose during lactation.

**Acknowledgements** This work was funded by the Department for the Environment, Food and Rural Affairs. G. Jaurena is grateful for scholarships provided by the British Council and Fundación Antorchas (Argentina).

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# Comparative study on amplification methods dedicated to gene profiling during trophoblast expansion in bovine embryos

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**Introduction** In bovine conceptuses, the trophoblast elongates rapidly from the 13th to the 19th day of pregnancy, while the embryonic disc differentiates to set up the primary germ layers and the axis of the fetus. Observations on pregnancies of cloned fetuses underline placental abnormalities which could originate from earlier gene deregulations occurring during this long pre-implantation period. To allow screenings with individual trophoblasts we used amplification protocols involving either PCR or in vitro transcription steps. For that purpose we used a bovine-specific macro-array starting from a cDNA library generated on early in vivo elongating conceptuses. RNAs from adult somatic tissues were used to compare hybridisation patterns generated with amplified and unamplified polyA+ probes.

**Material and methods** Ovaries and brain were collected on a Day 50 pregnant cow and immediately snap-frozen. RNA extractions were done using RNAPlus<sup>TM</sup> (Quantum Appligene) according to the manufacturer's instructions. PolyA+ was purified on dynabeads Oligo(dT)<sub>25</sub> (Dynal). Antisense RNA (aRNA) was generated starting from 1µg of total RNA as described by the MessageAmp<sup>TM</sup> aRNA kit (Ambion). 500ng polyA+ or aRNA were labelled with <sup>33</sup>P αdATP according to Decraene et al., 1999. Starting from 1µg of total RNA, cDNAs were generated by PCR amplification as described in Revel et al., 1995 and <sup>33</sup>P-labelled (500ng) with a random priming kit (Atlas<sup>TM</sup> SMART<sup>TM</sup> Probe Amplification kit, Clontech). For each protocol, 3 probes have been generated independently and each of these has been hybridised to 4 identical membranes. The membranes were hybridised (16h) and washed at 68°C according to Clontech's procedure. They were exposed to phosphoscreens (Amersham) and scanned after 7 days (Storm 760, Molecular Dynamics). Quantifications were done using ImaGene 5.1 (BioDiscovery).

**Results** Amplification protocols have been tested using either increasing in vitro transcription time (aRNA) or PCR cycle number (cDNA) to define optimised amplifications. Once defined (10h for aRNA synthesis; 12 cycles for the 1<sup>st</sup> and the 2<sup>nd</sup> PCR rounds), these protocols were used to generate three independent probes each. 3 polyA+ probes were generated too. Slot blot hybridisations (using endogenous genes) performed on polyA+ and amplified material were quantified, evidencing a good reproducibility among probe replicates. For each protocol and tissue, the hybridisation profiles have been compared, taking into account signal intensities 2 times above background. For both tissues, over 80% of the signals generated by aRNA or cDNA probes had the same address than signals generated with polyA+ probes. However, cDNA probes gave 64 to 71% of the polyA+ signals generated by brain and ovary probes, whereas aRNA probes gave only 36 to 38% of these signals.

**Conclusions** Although the polyA+ hybridisation pattern is more extensively reproduced with cDNA probes (64-71% identical spot positions), a statistical analysis of under/over/evenly expressed genes is now in progress to compare the expression profiles generated by polyA+, aRNA, and cDNA probes with both tissues. Depending on these results, cDNA or/and aRNA amplification protocols will be extended to embryonic material.

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## The study of reproductive failures in dairy cattle by metabolic profile test in Tabriz

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**Introduction** The deficiency of some blood elements and metabolites such as phosphorus, calcium, haematocrit, hemoglobin, glucose and protein, and also increased the urea and total protein in blood appears to have an important role to control reproductive failures in dairy cows. Recent studies showed that some metabolites and nutritional factors are responsible for dairy cows infertility (Mc Clure, 1994, Ramakrishna, 1996). The energy shortage in postpartum period led to decrease in luteinizing hormone secretion frequency and dynamic follicle diameter, however it increases interval calving\_time (Almeida, 1995). The objective of this study was to investigate the relation between blood metabolites and elements on reproductive failures in dairy cows.

**Materials and methods** For this study, 72 healthy and 72 affected cows were chosen as control and reproductive failure group, respectively. The affected cows were identified as their reproduction background and included repeat breeder, persistent corpus luteum (PCL), inactive ovaries and persistent follicles. Diagnosis of reproductive failures was evaluated by study of management data, rectal examination and measuring of plasma progesterone. The serum was prepared by taking a sample of 5 ml blood sampled from each cows. The concentration of elements and metabolites in blood were measured by spectrophotometry. The data were analyzed using SAS program and GLM model used to analysis of variance.

**Results** There was no significant difference ( $P>0.05$ ) in haematocrit and hemoglobin concentrations (Table 1) between healthy and affected cows. Also there is no difference between PCL and repeat breeder cows in the mentioned metabolites. However, cows affected by inactive ovaries and persistent follicles had a lower concentration of hematocrit and hemoglobin than others. The concentration of blood protein in cows with persistent follicle was less than healthy and PCL cows ( $p<0.05$ ). There were no significant differences ( $P>0.05$ ) in blood urea between healthy and inactive ovaries cows. The results showed that blood urea in the repeat breeder cows were significantly higher than healthy cows ( $p<0.05$ ). However, concentration of blood glucose in the healthy cows, persistent corpus luteum cows and repeat breeders was the same but, it decreased in inactive ovaries and persistent follicle cows ( $P<0.05$ ). The phosphorus concentration in healthy cows was higher than inactive ovary, persistent follicle and repeat breeders cows ( $P<0.05$ ). The calcium concentration in blood was contestant in all studied animals.

**Table 1** Composition of metabolites and elements in different statues in dairy cows

Items	Healthy	Inactive ovary	Persistent Corpus luteum	Repeat breeder	Persistent follicle
PCV%	32.77	29.81	32.58	24	28.36
Hb g/dl	10.92	9.9	10.81	11.3	9.45
Protein g/dl	7.3 <sup>a</sup>	7.03 <sup>ab</sup>	7.42 <sup>a</sup>	7.07 <sup>ab</sup>	6.5 <sup>b</sup>
Urea mg/dl	23.72 <sup>bc</sup>	22.81 <sup>bc</sup>	26.29 <sup>b</sup>	41.43 <sup>a</sup>	21.95 <sup>c</sup>
Glucose mg/dl	47.15 <sup>a</sup>	40.45 <sup>b</sup>	48 <sup>a</sup>	45.89 <sup>a</sup>	36.18 <sup>c</sup>
Phosphorus mg/dl	5.97 <sup>a</sup>	5.25 <sup>c</sup>	5.84 <sup>ab</sup>	5.12 <sup>c</sup>	5.45 <sup>bc</sup>
Calcium, mg/dl	8.33	8.2	8.34	8.56	8.39

Mean in every row with different letter have significant difference ( $p<0.05$ )

**Conclusion** Obtained results from this study showed that the incidence of low reproductive performance may associate with decrease of some blood metabolites and elements. Therefore the deficiency of nutrients supply could induce reproductive failures in dairy cows. These data suggested that it is possible to diagnose reproductive failures in dairy cows by testing metabolic profile in blood stream and so improve the rate of pregnancy in dairy cows.

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## Effects of four contrasting grassland-based milk production systems on dairy cow fertility

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**Introduction** High genetic merit dairy cows were managed on four contrasting grassland based systems of milk production over a three-year period (Ferris *et al.*, 2000). Each of the four systems were designed to incorporate a range of different approaches to increasing nutrient intakes. Key performance data from this study are presented in Table 1. The aim of this paper is to examine the effects of the four milk production systems on dairy cow fertility.

**Material and methods** During a three year period a total of 240 winter calving Holstein/Friesian dairy cows (80/year) were used in a study involving a comparison of four milk production systems (Systems 1 - 4). A total of 233 cow observations were obtained from the study, representing 58 observations for each of Systems 1 - 3, and 59 observations with System 4. The animals involved in the study had a mean PTA<sub>2000</sub> fat + protein of 31.1 kg, a mean lactation number of 1.9, and a mean calving date of 16 November. Each of the four systems examined incorporated contrasting feeding and management approaches, with each system designed to allow high merit dairy cows to achieve increased nutrient intakes: During the winter, animals on Systems 1 and 2 were offered high feed value grass silages (D-value, 740), supplemented with 6.0 kg/day of concentrate (crude protein, 286 g/kg DM) through an out-of-parlour feeding system, while animals on Systems 3 and 4 were offered medium feed value silages (D-value, 650), supplemented with 12.5 kg concentrate/day (crude protein, 213 g/kg DM), in the form of a complete diet. Animals on Systems 1 and 3 commenced grazing on 15 March, with these animals being offered a large daily herbage allowance during the summer (23.0 kg grass DM/cow, above 4.0 cm), supplemented with 0.5 kg concentrate/day. Animals on Systems 2 and 4 had a later turnout date (8 April), and during the summer were managed on a restricted herbage allowance (16.8 kg DM/cow, above 4.0 cm) with concentrates (mean, 3.9 kg/day) being offered to yield. All animals were bred by artificial insemination (AI), with AI commencing 8 December, and finishing 30 June the following year. No animal was inseminated or treated with fertility drugs before day 42 post-calving. System effects on animal performance and dairy cow fertility were analysed using Analysis of Variance.

**Results** While concentrate inputs increased from System 1 through to System 4 ( $P < 0.001$ ), lactation milk output was unaffected by system of milk production ( $P > 0.05$ ). System had no significant effect on any of the fertility parameters examined, although there was considerable variation between systems with a number of the parameters examined. For example, calving interval ranged from 379 days (System 3) to 400 days (System 1). The high calving interval recorded with System 1 can be attributed in part to a number of animals which experienced early embryonic death, and which subsequently got back in calf towards the end of the breeding season.

**Table 1** System effects on cow performance and fertility (proportional basis, unless stated otherwise)

	System 1	System 2	System 3	System 4	SEM	SIG
<b>Animal performance/lactation</b>						
Concentrate input (kg DM)	928	1388	1851	2277	34.8	***
Milk output (kg)	7868	8083	7721	8007	136.8	NS
<b>Fertility parameters</b>						
Interval to 1 <sup>st</sup> observed heat	46	46	42	47	2.9	NS
Animals observed in heat pre day 42	0.41	0.47	0.53	0.39	0.066	NS
Interval to 1 <sup>st</sup> service	68	65	63	67	2.6	NS
Conception to 1 <sup>st</sup> service	0.38	0.36	0.41	0.32	0.064	NS
Conception to 1 <sup>st</sup> and 2 <sup>nd</sup> service	0.64	0.55	0.62	0.66	0.064	NS
Number of services/cow	2.26	2.29	2.19	2.19	0.176	NS
Number of services/pregnancy	2.47	2.60	2.40	2.26		
Pregnancy rate during breeding season	0.91	0.88	0.91	0.97	0.036	NS
Calving interval (days)	400	389	379	386	6.7	NS

**Conclusions** Although the four systems examined involved very different levels of concentrate inputs, silages of different feed values, and very different grazing management regimes, system had no significant effect on any of the fertility parameters examined. This finding reflects the similar milk output and similar degree of body condition loss with each of the four systems, and highlights that animals managed on systems involving very different levels of concentrate inputs can achieve very similar levels of fertility. Nevertheless, while fertility levels in the study are similar to the national average, they are unacceptably low.

**Acknowledgments** This work was co-funded by DARDNI, AgriSearch, MDC and IFI Ltd

**Reference** Ferris *et al.* (2000) A comparison of four contrasting milk production systems for high genetic merit winter calving dairy cows in a grassland based production environment. BSAS Winter Meeting, Scarborough. P 12.

## The influence of GnRH treatment on the rate of reproductive development in bull calves

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**Introduction** Bull calves have an increase in Gonadotrophin Releasing Hormone (GnRH) pulse frequency early in life that is responsible for a short-lived rise in circulating levels of LH. It has been shown that bulls with a higher early rise in LH attain puberty at younger ages and have comparatively enhanced semen quality once they mature (Evans *et al.*, 1995). Furthermore testicular growth has been enhanced in calves by artificially inducing a premature increase in LH through administering GnRH i.v. every 2 hours between 4 and 6 weeks of age (Chandolia *et al.*, 1997).

**Objective** The objective of this study was to design a more practical treatment regime to improve testicular growth rates, leading to earlier ages at puberty.

**Method** All experimental work was conducted according to the Canadian Council of Animal Care as supervised by the local Animal Care Committee. Between 4 and 8 weeks, 2 groups of 6 bull calves received twice daily i.m. injections of either GnRH (120ng/kg) or Saline. Blood samples were collected through jugular cannulae every 15 min for 10 h at 4, 8, 14, 20, 26, 32, 38 and 44 weeks of age (at weeks 4 and 8, GnRH/saline was injected after the 5<sup>th</sup> and last blood sample). Every 2 weeks from 2 weeks of age until puberty, the scrotal circumference (s.c.) of each bull was measured. Puberty was assumed to have occurred when the s.c. reached 28 cm. Animals were electro-ejaculated every two weeks after they had reached a s.c. of 27.5 cm.

**Results** LH pulse frequency, pulse amplitude and mean levels were not shown to be significantly different between groups at any age. Mean LH levels and LH pulse frequency changed significantly over time ( $P < 0.001$ ) with a transient increase seen at 8 and 14 weeks in each group. LH pulse amplitude changed significantly over time ( $P < 0.001$ ) with high pulses evident at 20 weeks. Scrotal circumference increased at a faster rate in the GnRH treated calves than in saline treated calves ( $P < 0.05$ ) and from 22 weeks onwards GnRH treated animals had a significantly higher s.c. ( $P < 0.05$ ). GnRH treated bulls reached a s.c. of 28 cm earlier than control bulls ( $41.7 \pm 2.22$  weeks vs.  $47.0 \pm 0.45$  weeks;  $P < 0.05$ ). The earlier age of puberty following GnRH treatment was combined with a more rapid rise in semen quality in GnRH treated calves. The age at which an ejaculate was collected which contained  $>50$  million spermatozoa (with a minimum motility of 10%) occurred earlier in GnRH bulls than control bulls ( $45.0 \pm 0.86$  weeks vs.  $49.0 \pm 1.13$  weeks;  $P < 0.05$ ).

Measurement	Age of Control Bulls	Age of GnRH Treated Bulls	P value
Scrotal circ. of 28cm	$47.0 \pm 0.45$ weeks	$41.7 \pm 2.22$ weeks	$P < 0.05$
Ejaculate $>50$ million sperm	$49.0 \pm 1.13$ weeks	$45.0 \pm 0.86$ weeks	$P < 0.05$

**Conclusion** In conclusion, treatment of bull calves with GnRH between 4 and 8 weeks of age advanced the rate of reproductive development.

**Acknowledgements:** The authors would like to thank the farm staff at Goodale experimental farm, University of Saskatchewan, for care of the animals

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# Repeated fence-line ram exposure advances the timing and improves the synchrony of oestrus and lambing in ewes

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## Introduction

Introduction of a ram to anoestrous ewes induces an almost instantaneous rise in LH pulse frequency. This is commonly sufficient to overcome the seasonal suppression of the hypothalamic-pituitary axis and induce a synchronous first ovulation. This study investigates the application of repeated fence-line ram exposure during the beginning of the breeding season to synchronize oestrus and subsequent lambing in ewes.

## Materials and methods

During September, multiparous mule ewes were assigned to groups C (n=102) or R (n=102). Group R underwent repeated fence-line ram contact on Days 0 (September 10<sup>th</sup>), 17 and 34 of the experiment for a period of 24 hrs on each occasion. Group C were isolated from any ram contact for the duration of the experimental procedure. Ewes in groups C and R were mixed and raddled rams (n=10) were introduced for breeding on Day 51, 17 days after the last ram exposure of R ewes. Raddle marks were recorded daily to identify the timing and numbers of ewes bred during the first oestrous cycle and then recorded weekly for the subsequent 34 days.

## Results

### *Time of breeding and conception rates to first service*

Ram-exposed ewes were on average marked earlier than control ewes (P<0.001). At each daily observation the cumulative number of R ewes marked was consistently greater than control ewes until 14 days after ram introduction (at least P<0.05). The variance around the median time from ram introduction (RI) to marking was less for R than C ewes (Levene's test; P<0.01) indicating a greater degree of synchrony in the timing of first service within ram-exposed ewes. There was no significant difference in mean numbers of ewes conceiving to first service.

### *Lambing data*

Within those ewes lambing to first service, ram-exposed ewes had an earlier mean lambing date than control ewes (P<0.001). Within 10 days of the onset of lambing of each group, 67% of R ewes had lambed compared to only 52% of C ewes (P<0.05). The divergence between groups, in terms of the total numbers lambed, increased over time with 89% of R ewes lambed by day 13 compared to only 72% of C ewes (P<0.01). The disparity between groups was sustained until day 16 of lambing (at least P<0.05). Furthermore there was less variance around the median lambing date within ram exposed ewes (Levene's test; P<0.05) indicating a compaction of the lambing period of R ewes. There was no significant difference between numbers of lambs born between treatment groups.

Breeding data			Lambing data				
Group	Mean time from RI to breeding (days ± SE)	Total number of ewes marked by Day 7 after RI	Mean number of ewes conceiving to 1 <sup>st</sup> service	Mean number of days from RI to lambing (days ± SE)	Total number of ewes lambed by day 10 of onset of lambing	Total number of ewes lambed by day 13 of onset of lambing	Lambing percentage (lambs per ewe lambing to first service ± SE)
R	4.7 ± 0.4	77%	81	152.0 ± 0.5	67%	89%	2.20 ± 0.07
C	7.7 ± 0.5	50%	82	154.6 ± 0.5	52%	72%	2.07 ± 0.08
P value	P<0.001	P<0.001	P=0.832	P<0.001	P<0.05	P<0.01	P=0.226

**Conclusion** In conclusion periodic, repeated fence-line ram contact advanced the timing and improved the synchrony of oestrus in ewes during the breeding season. The observed compaction of the service period translated to a greater degree of synchrony at lambing and a concentration of the lambing period with no negative impact upon ewe fertility.

**Acknowledgements** We thank J. Wightman, D. Routledge and A Fogerty for care of the ewes. We also thank the Yorkshire Agricultural Society for their financial support.

## The effect of energy intake level, body condition score, and leptin on ovulation rate in fat-tailed ewes

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**Introduction** Ovulation rate (OR) is depended to energy intake level (EIL) and body condition score (BCS) in ewe (Gordon, 1997). There is a high correlation between BCS and plasma leptin concentration (PLC) in sheep (Blache *et al*, 2000). Corresponding to leptin is a metabolic stimulant for reproduction (Barb *et al*, 1999), it is possible that leptin is involved in the ovulation rate in ewe. Therefore, first experiment was conducted to study the effect of EIL on body weight (BW), BCS, OR and PLC in a long period, and second experiment was designed to investigate the effect of EIL and leptin administration on OR in a short period in fat-tailed Shal ewes.

**Materials and Methods** In the first experiment, 28 synchronized Shal ewes were assigned randomly to a body weight maintenance (M, n=14) or an energy-restricted (60% maintenance), body weight loss (L, n=14) treatment during 5 estrous cycles (10 weeks) in 2000. In the second experiment, 16 synchronized Shal ewes were assigned randomly into two groups and injected intravenously 1µg/Kg recombinant human leptin (Trt, n=8) or 1ml physiological saline (Con, n=8) for 4 days in 2001. Animals were fed a ration which provided 100% maintenance energy requirements. In trial 1, BW, BCS (0-5), and OR were determined in each estrous cycle. Heparinized blood samples collected from the jugular vein twice weekly to measure plasma progesterone concentration, and a day before estrous in each cycle to measure PLC. In trial 2, BW, BCS, and OR were determined and blood samples were collected before and after treatments. Plasma hormones concentrations were measured by double-antibody RIA. Differences in ovulation rate were compared by chi-squared test. The effect of treatment and of time and interaction were analyzed by repeated measures GLM for the BW, BCS, and PLC in the first experiment. Data of the BW, BCS, and PLC of the second experiment were analyzed by ANOVA.

**Results** In trial 1, Mean BW, BCS, and PLC significantly ( $p<0.01$ ) decreased in "L". There is positive correlation between BW and BCS ( $p<0.01$ ,  $r=+0.79$ ), between BW and PLC ( $p<0.01$ ,  $r=+0.50$ ), and between BCS and PLC ( $p<0.01$ ,  $r=+0.70$ ). In "L", OR significantly ( $p<0.05$ ) decreased when BCS had been later than 2, and PLC had been almost 2ng/ml. Estrous cycle and ovulation were stopped when BCS and PLC had been 1 and almost 1ng/ml, respectively (Table. 1). In second experiment, mean PLC was significantly increased ( $p<0.01$ ) in Trt, but there were not any significant differences in BW, BCS, and OR between two groups.

**Table 1** Mean ( $\pm$ S.E.M.) BW (Kg), BCS (0-5), PLC (ng/ml), and OR in ewes that were fed rations which provided 60% (L) or 100% (M) energy maintenance requirements during 10 weeks.

week		0	2	4	6	8	10
trait/group							
BW	L	45.14 <sup>a1</sup> $\pm$ 0.68	41.64 <sup>b</sup> $\pm$ 0.72	41.31 <sup>b</sup> $\pm$ 0.89	39.73 <sup>bc</sup> $\pm$ 0.97	37.96 <sup>c</sup> $\pm$ 0.75	35.31 <sup>d</sup> $\pm$ 0.62
	M	45.18 <sup>a</sup> $\pm$ 0.60	46.25 <sup>a</sup> $\pm$ 0.74	45.29 <sup>a</sup> $\pm$ 0.59	45.50 <sup>a</sup> $\pm$ 0.66	44.82 <sup>a</sup> $\pm$ 0.57	45.07 <sup>a</sup> $\pm$ 0.65
BCS	L	2.91 <sup>a</sup> $\pm$ 0.11	2.30 <sup>b</sup> $\pm$ 0.10	2.15 <sup>b</sup> $\pm$ 0.09	1.75 <sup>c</sup> $\pm$ 0.06	1.35 <sup>d</sup> $\pm$ 0.06	1.04 <sup>e</sup> $\pm$ 0.04
	M	2.95 <sup>a</sup> $\pm$ 0.11	2.91 <sup>a</sup> $\pm$ 0.10	2.98 <sup>a</sup> $\pm$ 0.06	2.96 <sup>a</sup> $\pm$ 0.08	3.00 <sup>a</sup> $\pm$ 0.12	2.98 <sup>a</sup> $\pm$ 0.07
PLC	L	3.54 <sup>ab</sup> $\pm$ 0.34	3.15 <sup>ab</sup> $\pm$ 0.17	2.87 <sup>bc</sup> $\pm$ 0.22	2.21 <sup>cd</sup> $\pm$ 0.40	1.97 <sup>d</sup> $\pm$ 0.22	1.16 <sup>e</sup> $\pm$ 0.53
	M	3.87 <sup>a</sup> $\pm$ 0.22	3.53 <sup>ab</sup> $\pm$ 0.20	4.03 <sup>a</sup> $\pm$ 0.07	3.33 <sup>bc</sup> $\pm$ 0.25	3.89 <sup>a</sup> $\pm$ 0.31	3.71 <sup>a</sup> $\pm$ 0.39
OR	L	1.29 $\pm$ 0.16	1.14 $\pm$ 0.02	1.31 $\pm$ 0.18	1.00 <sup>*</sup> $\pm$ 0.16	0.62 <sup>**</sup> $\pm$ 0.14	0.08 <sup>**</sup> $\pm$ 0.08
	M	1.36 $\pm$ 0.13	1.21 $\pm$ 0.15	1.29 $\pm$ 0.16	1.36 $\pm$ 0.13	1.43 $\pm$ 0.14	1.36 $\pm$ 0.13

<sup>1</sup>Values with different superscripts in each traits are significantly different ( $p<0.05$ ); \*  $P<0.05$ ; \*\*  $P<0.01$ .

**Conclusions** Results indicate that ovulation rate have a direct relationship with plasma leptin concentration in ewe, but there is not any evidence to show that leptin directly affects on ovulation rate. There is, however, a positive correlation between plasma leptin concentration with body weight, and with body condition score in fat-tailed ewes, similar to thin-tailed ewes (Blache, *et al*, 2000). These findings, also suggest that fat-tailed ewes are resistant to body energy changes.

**Acknowledgments** The Authors thank TMU and ASRI for financial support and the help of Prof. A. Nik-Khah, Dr. A. Niasari, and Dr. S.A. Mirhadi.

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## Effect of nutrition on endometrial progesterone receptor expression in ewes

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**Introduction** It has been postulated that undernourishment could affect embryo survival through changes in the uterine environment (Abecia et al., 1995). Moreover, we have shown that undernourished ewes had higher plasma progesterone (P4) concentrations and a lower endometrial content of P4 (Lozano et al., 1998), suggesting that this lower endometrial content could be due to a decrease in the content of endometrial progesterone receptors (PR). The aim of this study was to investigate the effect of low and high levels of food intake on PR in different endometrial cell types.

**Materials and Methods** Rasa Aragonesa ewes (liveweight: 56.7±1.85 kg) synchronized with intravaginal progestagen pessaries were randomly assigned at pessary withdrawal to a diet of 1.5 (Group H, n=13) or 0.5 times (Group L, n=13) the daily maintenance requirement (Lozano et al., 1998). On Day 5 and 10 of the second cycle following progestagen removal, ewes were euthanized, and pieces of endometrium were dissected and fixed by immersion in 4% paraformaldehyde. An immunohistochemical technique (avidin-biotin-peroxidase) was used to visualize PR immunostaining as described previously (Meikle et al., 2000) using a monoclonal mouse primary antibody (Zymed # 18-0172). PR expression was subjectively studied by 2 observers in 8 uterine compartments defined by cell type and location: caruncular and intercaruncular epithelium (CLE, ILE), superficial and deep glandular epithelium (SGE, DGE), superficial and deep intercaruncular stroma (SIS, DIS) and superficial and deep caruncular stroma (SCS, DCS). The staining of the nuclei was scored as being negative (-), faint (+), moderate (++) or intense (+++) and the proportion of the scale of staining of each field was expressed in a scale 0-10. The average staining was calculated as = 1 x n<sub>1</sub> + 2 x n<sub>2</sub> + 3 x n<sub>3</sub>, where n = proportion of cells per field exhibiting faint (1), moderate (2) and intense (3) staining. Average staining was analyzed using the mixed procedure (SAS), and the statistical model included the effect of observer, treatment, day, cell type and location and interactions between them.

**Results and Discussion** Staining of PR was seen exclusively in the nuclei of endometrial cells. Group L presented less intense staining (lower PR concentrations) than Group H on Day 5 of the cycle. However, this difference was not observed on Day 10 in any cell type (Table 1). Receptors are able to concentrate and retain the specific hormones in the target tissues, thus, the lower PR contents in Group L may explain our previous finding (Lozano et al. 1998) which showed that undernourished ewes had lower endometrial content of P4. We have not found reports linking PR uterine expression and nutrition, but a possible candidate is IGF-I: systemic IGF-I decrease in undernourished ewes (Thomas et al. 1990); IGF-I stimulates ER $\alpha$  function in a ligand-independent manner and ER $\alpha$  is in turn the main inductor for PR expression. Thus, a low nutritional level may result in lower amounts of endometrial PR, via the IGF-I/ER $\alpha$  pathways.

**Table 1** Average immunoreactivity in endometrial tissue on Days 5 and 10 of the oestrus cycle (oestrus=Day 0), in ewes fed 1.5 (Group H) and 0.5 times (Group L) the daily maintenance requirement (least square means  $\pm$  pooled s.e.).

Cell type	Compartment	Group L		Group H	
		Day 5	Day 10	Day 5	Day 10
Epithelial cells	ILE	1.4 $\pm$ 0.1 <sup>a</sup>	0.8 $\pm$ 0.1 <sup>b</sup>	1.8 $\pm$ 0.1 <sup>c</sup>	0.8 $\pm$ 0.1 <sup>b</sup>
	CLE	1.3 $\pm$ 0.1 <sup>a</sup>	0.6 $\pm$ 0.2 <sup>b</sup>	1.8 $\pm$ 0.1 <sup>c</sup>	0.8 $\pm$ 0.1 <sup>b</sup>
	SGE	1.5 $\pm$ 0.1 <sup>a</sup>	0.8 $\pm$ 0.1 <sup>b</sup>	1.9 $\pm$ 0.1 <sup>c</sup>	0.9 $\pm$ 0.1 <sup>b</sup>
	DGE	0.9 $\pm$ 0.1 <sup>a</sup>	1.0 $\pm$ 0.1 <sup>a</sup>	1.2 $\pm$ 0.1 <sup>b</sup>	0.9 $\pm$ 0.1 <sup>a</sup>
Stromal cells	SIS	0.8 $\pm$ 0.1 <sup>a</sup>	0.9 $\pm$ 0.1 <sup>a</sup>	1.1 $\pm$ 0.1 <sup>b</sup>	0.8 $\pm$ 0.1 <sup>a</sup>
	DIS	0.6 $\pm$ 0.1 <sup>ab</sup>	0.5 $\pm$ 0.1 <sup>ab</sup>	0.7 $\pm$ 0.1 <sup>b</sup>	0.4 $\pm$ 0.1 <sup>a</sup>
	SCS	0.6 $\pm$ 0.1 <sup>a</sup>	0.7 $\pm$ 0.2 <sup>a</sup>	1.2 $\pm$ 0.1 <sup>b</sup>	0.8 $\pm$ 0.1 <sup>a</sup>
	DCS	0.7 $\pm$ 0.1 <sup>a</sup>	0.8 $\pm$ 0.2 <sup>a</sup>	1.3 $\pm$ 0.1 <sup>b</sup>	0.9 $\pm$ 0.1 <sup>a</sup>

Different superscripts within the same row are significantly different P<0.05.

All Group H cell types showed more intense staining on Day 5 than on Day 10 in agreement with the known P4 downregulation of its own receptor observed in many species (Clark et al. 1992). In group L only three cell types showed a similar pattern (ILE, CLE, SGE) and we have no obvious explanation for this. Moreover, Group L had higher P4 plasmatic levels than Group H, which could have provoked a more pronounced downregulation. On the other hand, lower PR contents were found on Day 5 in this group (only ILE, CLE and SGE had an average staining  $\geq$  1), suggesting that the endometrium was less sensitive to P4 already at day 5 and thus, the inhibition was not observed.

**Conclusion** Results indicate for the first time that endometrial expression of progesterone receptors is affected in a temporal and spatial-specific manner by plane of nutrition.

**Acknowledgments** Project AGL2001/1817 from CICYT (Spain).

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# The effect of melatonin treatment during the seasonal anoestrus on the superovulatory response and embryo production of high-prolificacy Rasa Aragonesa ewes before culling

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**Introduction** The efficacy of melatonin implants inserted around the spring equinox to improve fertility and ovulation rate or litter size in Mediterranean ewes has been previously reported (Chemineau et al., 1996; Forcada et al., 2002a), indicating the ability of the hormone to regulate the hypothalamic activity (Viguié et al., 1995). Moreover, a direct effect of melatonin on corpora lutea and embryonic development has been also reported (Wallace et al., 1988; Abecia et al., 2002). The use of prolific Rasa Aragonesa (RA) ewes (a Mediterranean breed) before culling as embryo donors has been previously tested in the breeding season (Forcada et al., 2002b). The aim of this experiment was to improve embryo production during the seasonal anoestrus period in selected superovulated RA ewes at the end of their reproductive lives through the use of melatonin.

**Material and Methods** Thirty two mature (>8 years of age) RA ewes, with more than six lambing periods and selected for a high prolificacy within breed (mean litter size  $\geq 1.4$  lambs per lambing) were used. In early March, 17 ewes (M) received (Day 0) a single implant containing 18 mg melatonin (Melovine, Ceva Santé Animale, Libourne, France); the remaining ewes were considered as controls (C). On Day 24, oestrus was synchronized using intravaginal sponges containing 40 mg FGA. All ewes were superovulated with a total dose of 176 NIH-FSH-S1 units of NIADDK-oFSH-17 (Ovagen ICP-LTD Ltd., New Zealand) in 8 doses administered i.m. at 12-h intervals starting 72 h before sponge removal. Rams of proven fertility were placed with the ewes at pessary withdrawal, and ewes were checked for oestrus every 8 h. The embryos were collected via mid-ventral laparotomy 7 days after the onset of oestrus (recovery 1). Recovered embryos were evaluated under a stereo microscope at a magnification of 20-40X and classified by their stage of development and morphological appearance. Both blastocysts and compacted morulae were considered as viable (freezable or transferable). The same procedure was repeated on Day 80 after melatonin implantation (recovery 2). Proportion of ewes showing oestrus or ovulating was compared by chi-square, and ovulation rate and number and embryos quality by one-way ANOVA. The main effects were analysed using the GLM procedure for repeated measures.

**Results and Discussion** Considering both recoveries, percentage of ewes showing oestrous behaviour was 100%, with 96.8% M and 86.2% C ewes ovulating with functional corpora lutea. Number of early embryos (earlier than compacted morula) were significantly reduced by melatonin ( $P < 0.01$ ). Performances of C ewes decreased in recovery 2, although the great variability of the studied traits made difficult to show any statistical difference (Table 1). More than 3 viable embryos were obtained in each recovery period from melatonin-treated ewes induced to ovulate after oFSH treatment. Using the same breed during the breeding season, we have previously obtained four viable embryos per ovulated ewe (Forcada et al., 2002b).

**Conclusion** Results of this study indicate that melatonin implants could be an effective treatment to improve the mean number of recovered blastocysts (7 days after the onset of oestrus) during the seasonal anoestrus in Mediterranean ewes superovulated 80 days after implantation. Further experiments involving a higher number of animals are necessary.

**Table 1** Effect of melatonin treatment on the ovarian response and embryo production of mature Rasa Aragonesa ewes before culling. The synchronization-superovulatory treatments began 24 (recovery 1) and 80 (recovery 2) days after melatonin implantation (Mean $\pm$ s.e.).

	Melatonin-implanted ewes		Control ewes	
	Recovery 1	Recovery 2	Recovery 1	Recovery 2
corpora lutea	10.0 $\pm$ 2.2	11.1 $\pm$ 2.6	9.8 $\pm$ 1.8	10.7 $\pm$ 2.4
recovered ova	6.9 $\pm$ 1.5	5.4 $\pm$ 1.4	6.9 $\pm$ 1.4	6.4 $\pm$ 1.1
fertilized embryos	4.1 $\pm$ 1.3	4.3 $\pm$ 1.0	4.0 $\pm$ 1.2	4.0 $\pm$ 1.2
early embryos (earlier than compacted morula)	0.6 $\pm$ 0.5	0.3 $\pm$ 0.1 <sup>A</sup>	0.7 $\pm$ 0.2	1.5 $\pm$ 0.4 <sup>B</sup>
blastocysts (except hatched blastocysts)	2.5 $\pm$ 0.9	2.4 $\pm$ 0.7 <sup>a</sup>	2.3 $\pm$ 0.8	0.8 $\pm$ 0.5 <sup>b</sup>
viable embryos (compacted morulae and blastocysts –except hatched blastocysts)	3.1 $\pm$ 1.1	3.3 $\pm$ 0.8	3.3 $\pm$ 1.2	1.8 $\pm$ 1.0

Different superscripts in the same row indicate differences (a,b  $P < 0.1$ ; A,B  $P < 0.05$ )

**Acknowledgements** Project AGL2001/1817 from CICYT (Spain).

**References** Abecia et al., Vet Res Com 2002; 26: 151-158; Chemineau et al., INRA Prod Anim 1996; 9: 45-60; Forcada et al., Aust J Agric Res 2002a; 53: 167-173; Forcada et al., Livest Prod Sci 2002b; 66: 263-269; Viguié et al. Biol Reprod 1995; 52: 1114-1120.

# Nutritional effects on maternal blood metabolites and on outcome of pregnancy of dry season kidding Tswana goats.

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**Introduction** Reproductive wastage in terms of abortion, foetal loss and neonatal loss is one of the factors undermining goat productivity in Botswana. Abortion storms occur during the winter months, (Binta *et al.*, 1996) which coincide with the dry season. Though infectious causes were found in aborting goats, these accounted for a relatively small proportion of the total number of animals aborting (Binta *et al.*, 1998). Due to relatively few cases of abortions caused by infectious agents in Norwegian dairy goats, Hussain *et al.*, (1996) suggested that nutritional and environmental factors might be important. The aim of study was to evaluate the nutritional effects on blood metabolites and pregnancy outcome of Tswana goats under tropical conditions.

**Materials and methods** Control (C; 38.8±0.88kg n=19) and Supplemented (S; 39.8±0.90kg n=18) free ranching mature Tswana female goats were used. The animals were dosed for internal parasites before the start of the experiment Supplementary diet (106g/kg crude protein, 10.2MJ/kg ME) composed of 40% sorghum stover, 14% wheat bran, 42% maize grain, 2% molasses, 1.4% urea, 0.3% dicalcium phosphste and 0.3% salt. It was given to S at an average rate of 400g/animal/d except two weeks before to parturition when it was increased to 750 and C given 600g/animal/d. Blood was collected through jugular venipuncture two weeks before to parturition from five animals randomly selected from each group. This was analysed for total protein, globulin, albumin, Ca, P, Cu, Zn, urea, cholesterol, triglycerides, tetraiodothyronine (T4), triiodothyronine (T3) and cortisol. General linear models (GLM) procedure was used to determine the effects of supplementation on body weight and condition score, reproductive wastage and blood parameters. The effects of supplementation on pregnancy rate were evaluated by frequency analysis, using a Chi-square test.

**Results and discussion** Eighty-nine percent of S animals kidded including abortions while the value was 79 in C animals, the difference was not significant (P>0.05). Prolificacy was higher for S but was not significantly different (1.93±0.17 vs 1.64±0.18kid/doe; P>0.05) between S and C groups. Percentage total reproductive wastage was significantly lower in S than C groups (P<0.05). Exclusion of infectious causes from the analysis also resulted in a significant difference (P<0.05) in reproductive wastage between S and C. It appears that infectious agents were not a prime cause of reproductive wastage. Birth weights were similar (2.7±0.11 and 2.6±0.13kg; P>0.05) between S and C animals. Two weeks before parturition S weighed more, (43.9±1.3 vs 39.1±1.4kg; P<0.05) and had better body condition (2.83±0.16 vs 1.89±0.16; P<0.001) than C animals. At parturition S were still heavier (40.1±1.3 vs 35.1±1.3kg; P<0.05). Dam weights at parturition of S (r=0.617; P<0.001) and those of C (r=0.550, P<0.05) were positively correlated to kid birth weights. However, it was only S whose weights at parturition was significantly (r=0.756, P<0.001) correlated to birth weights of multiples. Most of maternal blood metabolites and cortisol levels were similar (P>0.05) between the groups (Table 1).

**Table 1** Blood parameter of Tswana goats at two weeks before parturition

Variable	S	C	SEM	P
Total protein (g/l)	66.64	61.02	3.39	NS
Albumin (g/l)	40.80	37.20	1.46	NS
Globulin (g/l)	25.84	25.22	2.01	NS
Calcium (mmol/l)	2.36	2.26	0.09	NS
Phosphorous (mmol/l)	1.98	1.76	0.14	NS
Copper (µmol/l)	18.16	20.36	2.54	NS
Zinc (µmol/l)	20.24	10.10	5.53	NS
Urea (mmol/l)	7.44	7.23	0.42	NS
Cholesterol (mmol/l)	1.65	1.14	0.14	*
Triglycerides (mmol/l)	2.41	2.12	0.58	NS
Haematocrits (%)	21.74	18.22	0.78	*
T3 (pmol/l)	5.54	4.30	0.30	*
T4 (nmol/l)	48.90	30.83	3.40	**
Cortisol (nmol/l)	24.74	18.30	5.00	NS

NS = P>0.05; \* = P<0.05; \*\* = P<0.01

A significant (P<0.05) difference was recorded on cholesterol, haematocrits, T3 and T4 (P<0.01) between the groups. Kid viability at birth contributed to reproductive wastage and difference in T4 may be a contributing factor but not cortisol. C as compared to S were undernourished, which was consistent with their low body condition, low body weight, low cholesterol and triglycerides levels two weeks before to parturition.

**Conclusion** Supplementary feeding of pregnant does grazing natural pasture during the dry season can offset the detrimental effects of maternal nutritional stress and therefore reducing reproductive wastage in goats.

**Acknowledgement** This study was funded by Botswana's Ministry of Agriculture.

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## Production of polyclonal antibody for ciprofloxacin detection in Brazilian livestock

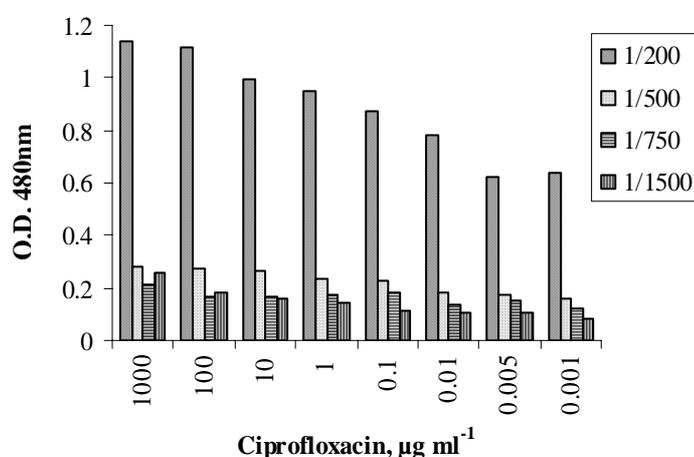
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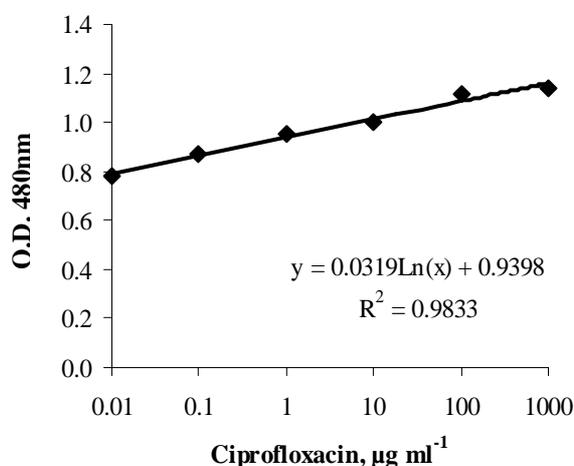
**Introduction** The antibiotic use to increase the animal production can result in residual concentrations in products as meat, eggs and milk, exceeding the acceptable doses for human consumption. In Brazil nowadays, 14 antibiotics are employed as feed additive, however there are about 121 drugs used in Veterinary Medicine that contain antibiotics in their formulation. Due to amplitude and frequency of the antimicrobial resistance, it can be considered an emergent and significant question for the public health and an alert for the necessity of a global control about the use of those antibiotics. The immunoassays allow the diagnosis of these antibiotics with accessible costs and presenting good results. The aim of this work was to produce polyclonal antibodies for ciprofloxacin detection using ELISA.

**Material and methods** Four New Zealand female rabbits were used to obtain the polyclonal antibodies in sequential immunization every 15 days, for 6 weeks (Duarte et al., 2002) with 40 $\mu$ g antibiotic molecule (ciprofloxacin) bound with bovine serum albumin (BSA). Before every immunization, blood samples were taken from the marginal vein of the rabbit ear, centrifuged and the sera were frozen. The sera were then used for titillation through indirect test of ELISA (“enzyme-Linked Immunosorbent Assay”), type “Plate Trapped Antigen” (PTA-ELISA) (Crowther, 1995) in order to detect the best concentration of the serum and the required antigen to sensitise the plates. Alkaline phosphatase and horseradish peroxidase were used as enzyme to develop the reactions in the plate. The spectrum count was realized at 480 nm in Bio Rad mod. 550. Data were collected and analysed with Packard System software. Data with three repetitions and with the mean superior to three times the negative control were considered positive reactions (Sutula et al., 1986)

**Results** Usually serum titre increase every new immunization, however, in this experiment the best serum titre was obtained from the female rabbit serum collected on the second week. The reason for this result was probably the animal variability, which may decrease the titre due to immunology tolerance development. The study of the serum dilution is showed on Figure 1. The highest spectrum count was obtained when 1/200 serum dilution was applied. In Figure 2, the linear regression between the spectra count and the ciprofloxacin doses using 1/200 serum dilution resulted in a proper quantification of the antibiotic for the calibration curve obtained.



**Figure 1.** Curves of ciprofloxacin quantification using polyclonal antibodies in different dilutions, as shown on legend



**Figure 2.** Linear regression of ciprofloxacin doses detected by polyclonal antibodies

**Conclusion** The results presented in O.D. at 480 nm made clear that the antibodies showed satisfactory titre for immune assay utilization to detect ciprofloxacin residues in livestock. The use of other kinds of floxacins, specially in Brazil such as norfloxacin, raxitrimicin, ofloxacin and enrofloxacin can be done cross reacting those antibodies with these molecules.

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# The influence of birth order and duration of farrowing on concentrations of metabolites in the umbilical cord blood of newborn piglets

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**Introduction.** It was the purpose of this study to ascertain whether concentrations of glucose (GLU), urea and non-esterified fatty acids (NEFA) in blood collected from the umbilical cords of newborn piglets vary according to the position of the piglet in the birth order of a litter. Umbilical cord blood is representative of the piglets status at the time of birth. It would therefore be advantageous to know whether blood withdrawn from the umbilical cord of one piglet is representative of the litter in terms of these metabolites. This study was therefore designed to test the hypotheses that position in the birth order, and time of birth relative to delivery of the first piglet in a litter, will influence GLU, urea and NEFA concentrations in umbilical cord blood.

**Materials and methods.** The farrowings of nine multiparous sows (mean parity 3), housed in conventional indoor farrowing crates, were attended. On delivery of each individual piglet, blood was first manually withdrawn from the umbilical cord into blood tubes and immediately centrifuged at 2688g. The serum was then decanted and frozen at -20°C for subsequent analyses. Time of birth was recorded relative to delivery of the first piglet in each litter and piglets were weighed and ear tagged for individual identification. Mean litter size was 12.2, SEM 0.47. GLU and urea concentrations were measured using ‘Infinity reagent’ obtained from Sigma Chemicals Ltd. (Poole, Dorset) on the Cobas MIRA analyser (Roche Diagnostics). NEFA concentrations were determined using the WAKO test kit (Alphalabs), again on the Cobas MIRA. Data were analysed in Minitab 12.2 using a one way analysis of variance to look at the effects either of birth order or sow on concentrations of the metabolites discussed. Linear regression analysis was used to determine any relationship between time of birth relative to delivery of the first piglet and metabolite concentrations.

**Results.** Piglet position in the litter birth order had no influence on the concentrations of GLU, urea or NEFA in blood sampled from its umbilical cord (Table 1). However metabolite concentrations did differ significantly between the litters of individual sows ( $P < 0.05$ , Table 2). There was no relationship between the time of birth relative to delivery of the first piglet in a litter and metabolite concentrations in the umbilical cord blood (Table 3).

**Table 1.** Effect of piglet position in litter birth order on concentrations of glucose, urea and non-esterified fatty acids in the umbilical cord blood.

	Piglet position in birth order														sem	Sig.
	1	2	3	4	5	6	7	8	9	10	11	12	13	14+		
GLU mmol/l	4.27	4.07	3.44	3.96	5.09	4.94	3.70	3.57	4.57	3.09	3.28	3.56	4.50	3.36	1.27	ns
Urea mmol/l	5.74	5.44	4.59	4.91	7.91	6.09	4.48	4.77	6.89	4.10	4.17	5.05	5.25	3.73	3.58	ns
NEFA meq/l	0.14	0.12	0.14	0.13	0.13	0.13	0.13	0.12	0.12	0.13	0.13	0.12	0.12	0.13	0.02	ns

**Table 2.** Average metabolite concentrations in the umbilical cord blood from litters of individual sows.

	Sow ID										sem	Sig.
	A	B	C	D	E	F	G	H	I			
GLU mmol/l	3.72	3.35	5.70	2.94	4.28	4.15	3.58	3.68	4.02	1.12	P<0.01	
Urea mmol/l	5.07	5.10	5.55	3.75	6.14	9.66	2.96	3.78	4.50	2.77	P<0.001	
NEFA meq/l	0.12	0.14	0.13	0.13	0.14	0.12	0.13	0.14	0.12	0.01	P<0.05	

**Table 3.** Relationship between time of birth (minutes) relative to delivery of the first piglet in a litter, with metabolite concentrations in umbilical cord blood.

	Regression equation	R-squared (%)	Sig
GLU mmol/l	Y=4.39-0.00447x	2.4	ns
Urea mmol/l	Y=5.03+0.0116x	2.3	ns
NEFA meq/l	Y=0.133-0.000070x	3.9	ns

**Conclusion.** These results indicate that an individual piglet may be considered representative of the litter in terms of glucose, urea and non-esterified fatty acid concentrations found in the umbilical cord blood, irrespective of its position in the litter birth order or the duration of farrowing. It is clear however, that the sow has a significant influence on metabolite concentrations within litter and this is likely to be linked to her metabolic state.

## The influence of n-6 and n-3 polyunsaturated fatty acids on eosinophils numbers in the gut of milk or milk replacer fed calf

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**Introduction** Infection by parasites is a major cause of production losses and mortality in young calves. The problem is most prevalent during the first grazing season (Armour, 1989). Eosinophils are important cellular mediators in immunity to gastrointestinal parasites (Baker et al, 1993), but during an extreme hypersensitivity immune response, degranulating eosinophils may lead to tissue pathology which may favour parasite survival (Miller, 1996). Dietary polyunsaturated fatty acids (PUFA) influence the fatty acid composition of immune tissues and cells (Jaffrey, 1998). n-6 and n-3 fatty acids are known to influence various components of immune response via eicosanoid dependent or independent mechanisms which influence the relative proportions of Th<sub>1</sub> and Th<sub>2</sub> cytokines, including IL-5 an important regulator of eosinophils maturation and recruitment. This study was carried out to establish the extent to which dietary n-6 or n-3 PUFA source affects the numbers of eosinophils in the gut of calves.

**Materials and Methods** Forty male Jersey calves were allocated at birth to 7 treatment groups. Group 1; 4 calves, fed colostrum according to the normal farm practice and slaughtered when 4 days old. Groups 2-7 (n=6), were fed colostrum for 4 days and were then allocated to be fed milk or milk replacer (MR) without supplement oil (Groups 2 and 3), with 25g fish oil/day (Groups 4 and 5) or with 25g of a mixture (50:50 w/w) of palm / rapeseed oil (PRO) (Groups 6 and 7) for a further 21 days. 30-mg  $\alpha$ -tocopherol acetate/kg PUFA was given to all calves receiving the oil supplement. Calves were slaughtered after fasting for 12 hours. Samples of abomasum (ABO), duodenum (DD) and terminal ileum (TI) were obtained for determination of eosinophil numbers. Data was analysed by ANOVA using the General Linear Model (GLM) (Minitab 13.0, Minitab, Inc, PA. USA). Significant differences were reported at p<0.05.

**Results** Eosinophil numbers were markedly higher in the terminal ileum (32±0.5) than in the duodenum (21±0.5) and were markedly lower in the abomasal tissue. Animals in group 1 had significantly fewer eosinophils (p<0.01) compared to other groups. Fish oil given in whole milk or MR lead to a significant reduction (p<0.05) in the number of eosinophils in the duodenum and terminal ileum when compared to PRO given in milk or MR (Groups 4 vs 6 and 5 vs 7). In the duodenum and terminal ileum, milk fed calves, (group 2, 4, and 6) tended to have lower number of eosinophils when compared to MR fed calves (groups 3, 5 and 7) irrespective of oil supplements.

**Table 1** Effect of n-6 and n-3 PUFAs on eosinophil numbers in the abomasum, duodenum and terminal ileum of calves fed milk or milk replacer (mean cells/10 areas of view).

Sample	Group 1 Colostrum	Group 2 Milk	Group 3 MR	Group 4 Milk+FO	Group 5 MR+FO	Group 6 Milk+PRO	Group 7 MR+PRO
ABO	3.4 <sup>a</sup> ± 1.1	3.8 <sup>b</sup> ± 0.8	3.4 <sup>a</sup> ± 0.9	3.6 <sup>a</sup> ± 0.9	4.2 <sup>b</sup> ± 0.9	4.9 <sup>b</sup> ± 0.8	4.5 <sup>b</sup> ± 0.8
DD	7.1 <sup>a</sup> ± 1.9	14.6 <sup>c</sup> ± 1.3	27.0 <sup>e</sup> ± 1.5	12.9 <sup>b</sup> ± 1.5	20.1 <sup>d</sup> ± 1.5	25.0 <sup>d</sup> ± 1.4	31.1 <sup>e</sup> ± 1.4
TI	12.0 <sup>a</sup> ± 2.3	29.9 <sup>b</sup> ± 1.6	36.2 <sup>c</sup> ± 1.8	21.7 <sup>d</sup> ± 1.8	28.8 <sup>b</sup> ± 1.8	29.0 <sup>b</sup> ± 1.7	43.6 <sup>e</sup> ± 1.7

Values are means ± SEM. Means with different superscripts on the same row are significantly different (P<0.05)

**Conclusion** The trend towards a greater number of eosinophils in the terminal ileum may have no significance but could suggest a site-specific role for these cells. MR appears to have some eosinophilic properties, since feeding MR irrespective of oil supplement tended to increase the number of eosinophils. Fish oil had the effect of reducing the number of eosinophils in the gut, irrespective of the liquid medium used. Overall, fish oil tended to counteract the eosinophilic property in MR. Feeding PRO, an n-6 PUFA source, tended to increase or maintain the number of eosinophils observed with feeding MR alone.

**Acknowledgements** Nelson Muturi is an Aga Khan Foundation Scholar.

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# Influence of dietary fatty acids on the fatty acid composition of gut mucosa in calves during the first 3 weeks of life

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**Introduction** The balance between pro and anti-inflammatory elements of the immune system can be influenced by provision of specific dietary polyunsaturated fatty acids (PUFA) (Calder, 1998). The gut associated lymphoid tissue (GALT) is the largest immune organ and an important regulator of tolerance and sensitivity to dietary and environmental antigens in the gut (Harbige and Fisher, 2001). Changing the fatty acid composition of the gut mucosa could influence the immune function of the GALT. This study was carried out to establish the extent to which dietary n-3 or n-6 PUFA-rich oil supplements could change the fatty acid composition of gut mucosa of the neonatal calf.

**Materials and methods** Twenty-eight, male Jersey calves were allocated at birth to 3 treatment groups: Group 1; received colostrum and was slaughtered at day 4. Group 2; received 25 g of fish oil per day, Group 3; received 25 g of a binary mixture of palm/rapeseed oil (50:50 w/w) per day. 30-mg  $\alpha$ -tocopherol acetate/kg PUFA was added to the milk supplement. Calves were slaughtered 21 days after allocation to the oil supplements, following a 12-hour fast. Gut mucosa was obtained from the small intestine and lipids present were extracted using the Folch method as described by Christie (1982). Fatty acid methyl esters were analysed by gas-liquid chromatography. Data were analysed by analysis of variance (ANOVA) (Minitab 13.0 Minitab, Inc, PA, USA).

**Results** The fatty acid composition of the gut mucosa was significantly influenced by the oil supplements. Compared to the colostrum fed calves, fish oil (n-3), lead to a significant increase in the proportions of C14:0, C18:0, C20:5n-3 C22:5n-3 and C22:6n-3, whereas the proportions of C18:1n-9, C20:4n-6 and  $\Sigma$ MUFA decreased significantly. In contrast feeding the n-6 PUFA-rich oil supplement significantly decreased the proportions of C14:0, C16:0, C18:1n-9, and  $\Sigma$ MUFA, whereas there were significant increases in C18:2n-6, C18:3n-3, C22:5n-3 and C22:6n-3. There was an increase in  $\Sigma$ SFA, and  $\Sigma$ n-3 in the n-3 group but not in the n-6 group and the increase in  $\Sigma$ PUFA and  $\Sigma$ n-6 was greater in the n-6 group than the n-3 group. These differences are reflected in the significant increase in P/S ratio in the n-6 group and the significant decrease in n6/n3 ratio in the n-3 group.

**Table 1** Comparison of fatty acid composition of gut mucosa from calves fed colostrum only, n-3 enriched fish oil or a binary mixture of palm and rapeseed oil during the 21-day feeding trial (g fatty acid/kg total fatty acids)

Fatty acids	Colostrum (n=4)	n-3 (n = 12)	n-6 (n = 12)
C14:0	22.7 <sup>b</sup> ± 8.7	37.8 <sup>c</sup> ± 4.8	20.2 <sup>a</sup> ± 4.8
C16:0	244.0 <sup>b</sup> ± 17.2	251.4 <sup>b</sup> ± 9.5	204.7 <sup>a</sup> ± 9.5
C18:0	143.4 <sup>a</sup> ± 10.8	170.4 <sup>b</sup> ± 6.0	156.7 <sup>a</sup> ± 6.0
C18:1n-9	318.2 <sup>c</sup> ± 20.4	227.8 <sup>a</sup> ± 11.3	268.5 <sup>b</sup> ± 11.3
C18:2n-6	83.0 <sup>a</sup> ± 22.0	108.6 <sup>a</sup> ± 12.2	155.2 <sup>b</sup> ± 12.2
C18:3n-3	6.5 <sup>a</sup> ± 1.1	6.6 <sup>a</sup> ± 0.6	10.5 <sup>b</sup> ± 0.6
C20:4n-6	39.1 <sup>b</sup> ± 5.5	24.3 <sup>a</sup> ± 3.1	39.1 <sup>b</sup> ± 3.1
C20:5n-3	2.3 <sup>a</sup> ± 0.2	14.6 <sup>b</sup> ± 1.5	3.0 <sup>a</sup> ± 1.5
C22:5n-3	9.9 <sup>a</sup> ± 1.8	10.7 <sup>b</sup> ± 1.0	10.6 <sup>b</sup> ± 1.0
C22:6n-3	2.7 <sup>a</sup> ± 0.5	8.5 <sup>c</sup> ± 0.8	4.2 <sup>b</sup> ± 0.8
$\Sigma$ SFA	425.1 <sup>a</sup> ± 23.2	487.4 <sup>b</sup> ± 12.9	399.9 <sup>a</sup> ± 1.2
$\Sigma$ MUFA	344.1 <sup>c</sup> ± 22.1	245.9 <sup>a</sup> ± 12.2	283.6 <sup>b</sup> ± 12.2
$\Sigma$ PUFA	145.9 <sup>a</sup> ± 29.4	175.2 <sup>b</sup> ± 16.4	225.6 <sup>c</sup> ± 16.4
$\Sigma$ n-6	123.7 <sup>a</sup> ± 26.6	134.0 <sup>a</sup> ± 14.7	196.6 <sup>b</sup> ± 14.7
$\Sigma$ n-3	22.1 <sup>a</sup> ± 5.4	41.2 <sup>b</sup> ± 2.9	29.0 <sup>a</sup> ± 2.9
P/S ratio	0.3 <sup>a</sup> ± 0.08	0.4 <sup>a</sup> ± 0.05	0.6 <sup>b</sup> ± 0.05
n-6/n-3 ratio	5.7 <sup>b</sup> ± 0.6	3.4 <sup>a</sup> ± 0.3	6.7 <sup>b</sup> ± 0.4

Values are means ± SEM. Means with different superscripts on the same row are significantly different ( $P < 0.05$ )

**Conclusions** Fish oil in the n-3 group and PRO in the n-6 group clearly influenced the fatty acid composition of gut mucosa. This may have consequences for tissue eicosanoid profiles. n-3 derived eicosanoids are anti-inflammatory while n-6 are known to be more pro-inflammatory elements of the immune system (Calder, 1998), thus the observed changes in the fatty acid profile and the n-6/n-3 ratio of the gut mucosa in the n-3 group may have consequences on the immune development and functionality of the GALT.

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## Effect of dietary polyunsaturated fatty acids on *ex-vivo* lymphocyte stimulation response to *Cooperia onchophara* L3 antigen in calves

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**Introduction** Dietary polyunsaturated fatty acids (PUFA) are known to influence the fatty acid composition of immune and inflammatory cell membranes (Yaqoob et al, 1995). Changing the fatty acid composition and the n-6/n-3 PUFA ratio of cell membranes has been reported to have profound effects on immune cell functionality (Blok et al, 1996). Immune responses to *Cooperia onchophara*, a nematode parasite that infects calves, are usually slow to develop and inappropriate. The aim of this experiment was to establish the extent to which supplementation of pre-ruminant calves with an n-6 or n-3 PUFA source may influence *ex-vivo* lymphocyte response to an L3 *C onchophara* antigen.

**Materials and Methods** Forty male Jersey calves were allocated at birth to 7 treatment groups. Group 1; 4 calves, fed colostrum according to the normal farm practice. Calves were slaughtered when 4 days old. Groups 2-7 (n= 6), were fed colostrum for 4 days and were then allocated to milk (M) or milk replacer (MR) without supplement oil (groups 2 and 3), with 25g fish oil/day (groups 4 and 5) or with 25g of a binary mixture (50:50 w/w) of palm / rapeseed oil (PRO) (groups 6 and 7) for a further 21 days. 30-mg  $\alpha$ -tocopherol acetate/kg PUFA was given to all the calves receiving the milk supplement. Calves were slaughtered after being fasted for 12 hours. Peripheral blood, mesenteric lymph node and spleen were collected under sterile conditions for lymphocyte harvesting. Lymphocytes were obtained by the method of (Schalling et al, 1998) and prepared for proliferation by the method of Boyum (1968). *C onchophora* L3 antigen was prepared by the method described by Huntley et al (1998). Lymphocyte stimulation index was obtained using the formula SI= Proliferation with mitogen/antigen divided by proliferation in unstimulated controls. Data was analysed by ANOVA, General linear model (Minitab, 13.0.PA.USA). Significant differences were reported at  $p<0.05$ .

**Results** Lymphocyte proliferation response to *C onchophara* L3 antigen was observed in PBMC, MLN and spleen lymphocytes in all of the groups. Group 1 had a significantly lower lymphocyte stimulation response ( $p<0.05$ ) when compared to all other groups. Feeding fish oil lead to a significantly lower response in PBMC, MLN and spleen lymphocytes in the milk fed calves compared to those given milk with additional PRO (groups 4 vs 6). Similarly adding fish oil to the milk replacer lead to a significant decrease ( $p<0.05$ ) in PBMC, MLN and spleen lymphocyte responses compared to addition of PRO (group 5vs 7). There is also some suggestion that compared to whole milk, the lymphocyte response in calves fed milk replacer tended to be higher in PBMC and MLN irrespective of oil supplements.

**Table** Effect of dietary polyunsaturated fatty acids on *ex-vivo* lymphocyte stimulation response to *Cooperia onchophara* L3 antigen in milk or milk replacer fed calves.

	Lymphocyte stimulation index (LSI)						
	Group 1 Colostrum	Group 2 M	Group 3 MR	Group 4 M+FO	Group 5 MR +FO	Group 6 M +PRO	Group 7 MR+PRO
PBMC	1.4 <sup>a</sup> ± 0.7	3.4 <sup>b</sup> ± 0.9	4.3 <sup>c</sup> ± 0.9	3.5 <sup>b</sup> ± 0.6	4.1 <sup>c</sup> ± 0.6	4.3 <sup>c</sup> ± 0.6	4.7 <sup>d</sup> ± 0.4
MLN	2.6 <sup>a</sup> ± 0.6	3.4 <sup>c</sup> ± 0.5	3.7 <sup>c</sup> ± 0.9	3.2 <sup>b</sup> ± 0.9	4.0 <sup>d</sup> ± 0.5	3.9 <sup>c</sup> ± 0.4	4.4 <sup>c</sup> ± 0.5
SPLEEN	1.5 <sup>a</sup> ± 0.3	3.3 <sup>b</sup> ± 0.6	3.4 <sup>b</sup> ± 0.6	4.0 <sup>c</sup> ± 0.5	4.1 <sup>c</sup> ± 0.5	4.7 <sup>d</sup> ± 0.4	4.8 <sup>e</sup> ± 0.4

Values are means ± SEM. Means with different superscripts on the same row are significantly different ( $P<0.05$ )

**Discussion** The responses in the day 4 old calves fed only colostrum (Group 1) is indicative of an age related hyporesponsiveness. Fish oil, an n-3 PUFA source appears to reduce the lymphocyte responses, while feeding an n-6 PUFA source (PRO) appears to increase the lymphocyte response in this study. n-3 PUFA derived eicosanoids have different potencies to those derived from n-6 sources and changing the ratio of these PUFA appears to influence lymphocyte proliferative responses in the calf. This could be used as a strategy to optimise the immune response to gut parasite infection, since immune activation in the gut causes some tissue damage and has a metabolic cost to the host.

**Acknowledgements** Nelson Muturi is an Aga Khan Foundation Scholar.

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## The effect of Lycopene, a carotenoid with strong antioxidant properties, and a Nutraceutical mix on the performance and immune function of weaned pigs.

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**Introduction** With the proposed ban on antibiotic growth promoters it is becoming increasingly important to provide diets to young pigs which enhance the long term health. Alternative strategies to promote health include organic acids, herbal products and natural antioxidants. These products have various proposed modes of action including improved liver function, removal of reactive oxygen species and the enhancement of the immune function. The objective of this experiment was to measure the effect of a commercial herbal mixture and Lycopene on the performance and immune function of weaned pigs.

**Material and methods.** 96 PIC Camborough 15 x PIC225 pigs were selected when they were weaned at 28 days of age from the Harper Adams Pig Unit. The health status of the unit was PRRS and EP negative and PMWS positive. The pigs were randomly allocated to one of three treatments with eight replicates of four pigs per pen. The treatments were A) Basal dietary regime, B) Basal diet plus a dietary supplement (Supplied by Frank Wright Ltd.) containing lycopene, herbs, oils, antioxidants and digestive enhancers and C) Basal diets plus lycopene at the same inclusion as treatment B. The basal diets consisted of a creep diet (DE 16.7 MJ/kg, CP 23.1%, Lys 1.7%) days 0-11, a link diet (DE 15.4 MJ/kg, CP 21.4% and Lys 1.5%) days 11-34, and a standard grower diet (DE 14.5 MJ/kg, CP 21%, Lys 1.4%) days 34-55. The test ingredients were included in the creep and link diets only and all diets were fed *ab-lib*. The pigs were housed in 24 fully slatted pens, in environmentally controlled flat decks from weaning to day 39. They were then moved and mixed within treatment to solid floored grower accommodation of 4 pigs per pen. Live weights and feed intake were recorded at regular intervals. Faecal samples were assessed for consistency at weaning and on day 11 on a scale of 1-5. Forty-eight pigs were blood sampled at weaning (day 0), day 14, 28, 33 and 56. Serum total antioxidant status was measured by Randox's TAS kit. On day 33, production of gamma-interferon (IFN $\gamma$ ) by isolated peripheral blood mononuclear cells (PBMC) following 16 hours cultured with concanavalin A was measured by ELISA (Tridelta ltd). Serum haptoglobin concentration was assessed by a haemoglobin binding assay on day 55 to coincide with likely Post-weaning Multisystemic Wasting Syndrom (PMWS).

**Results.** Overall performance was good across all pigs with no significant treatment differences in growth. There was a significant correlation between the faecal score measured on day 11 and subsequent daily-gain and FCR. Gain (d11-27) = 0.405 + 0.081 Faecal Score (R<sup>2</sup> 0.24, p=0.009). FCR (d11-27) = 1.409 - 0.083 Faecal Score. (R<sup>2</sup> 0.16, p=0.031). The mean treatment faecal scores were reflected in the post weaning food efficiency trends. There were no treatment effects on serum total antioxidant status. The haptoglobin responders (>3 mg/ml) at day 55 were highest in the control group 53%, 31% & 40% for treatments A,B & C respectively. IFN $\gamma$  production by PBMC's was not normally distributed and required a log transformation. There were no correlations between the immunological measurements and performance. However there was a negative correlation between the faecal score at day 11 and IFN $\gamma$  production by PBMC's (-57.9, R<sup>2</sup> 0.10, p=0.025). The incidence of PMWS could not be analysed using chi square analysis.

**Table 1** Effect of Herbal and Lycopene supplementation on the performance of weaned pigs.

	Basal	Nutraceutical	Lycopene	s.e.d.	P
Gain 0-11(g/d)	391	402	393	31.6	NS
Gain 11-39(g/d)	676	679	667	28.1	NS
Gain 39-55(g/d)	649	759	677	60.9	NS
FCR 0-11	0.93	0.93	0.97	0.051	NS
FCR 11-39	1.52	1.52	1.57	0.044	NS
FCR 39-55	2.134	1.932	2.244	0.124	0.059
Haptoglobin D55 mg/ml	3.72	2.43	2.75	0.663	NS
IFN $\gamma$ Conc (pg/ml)	142	111	161		
Log 10 IFN $\gamma$ (pg/ml)	2.01	1.92	2.00	0.154	NS
Faecal Score D11	2.875	3.031	2.781	0.1904	NS
PMWS/PDNS Deaths	3	0	0		

**Conclusion** There is a clear correlation between gut health as measured by faecal score, and both performance and physiological function in the weaner pig. The trends in all the physiological measurements suggest that the inclusion of dietary antioxidants can effect performance and health.

## Molecular detection of tick-borne pathogens in small ruminants

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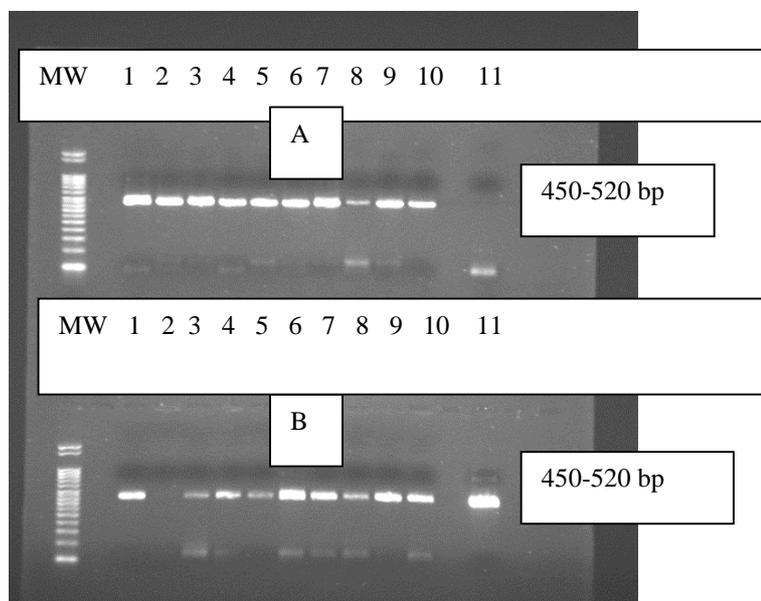
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**Introduction** Ticks and tick-borne pathogens are responsible for mortality and morbidity in livestock, wildlife, pets and humans in many countries. However, detection is difficult with multiple infection cases and low infection rates in carrier animals. We used a macro-array-based method to simultaneously detect tick-borne pathogens.

**Materials and Methods** DNA samples were extracted from sheep blood samples collected from three regions in Italy not showing any clinical reactions. One 18S rRNA-based PCR targeted *Theileria/Babesia* species (Georges et al, 2001) while a 16S rRNA-based PCR detected bacteria (Bekker et al, 2002). Then a reverse line blot hybridisation was performed with family and species-specific DNA probes (Georges et al, 2001).

**Results** 78.33% of small ruminant blood samples (60 animals) were infected with protozoa pathogens from the genera *Theileria* or/and *Babesia* (see Figure 1), while 0.2% of the same animals were infected with bacteria. The Reverse line blot hybridisation method showed that non-*Theileria annulata* species were present in these animals. The previous cattle-related probes did not cross react with the small ruminant pathogens highlighting that other *Theileria* and *Babesia* species are present.



**Figure 1** Gel electrophoresis analysis of PCR amplification for protozoa. Set A, lanes 1 to 10: DNA from sheep blood samples. Lane 11: negative control. Lane MW: molecular marker. Set B, lanes 1 to 10: DNA from sheep blood samples. Lane 11: positive control with *Theileria buffeli*.

**Conclusions** These results are showing a very high prevalence of *Theileria/Babesia* species that needs to be specifically identified. Therefore further tests, including DNA sequencing, will be carried out to compare the pathogen DNA and reference DNA sequences in international DNA banks.

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# The influence of farriery regimens and dietary management on equine predisposition to white line disease

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**Introduction** The biodeteriorative condition commonly known as white line disease (WLD) tends to be most prevalent in parts of the world where warm humid conditions prevail e.g.USA, Japan. However, during the last decade, the UK has experienced warmer and significantly wetter summers that have led to a significant increase in the incidences of the disease amongst the British equine community. Although a variety of factors (e.g. environmental, nutritional, microbial, mechanical (see Woodall, 2002 for review)) have been implicated in the onset of the condition, scientists are still a long way from understanding the aetiology of the disease. There can be no doubt that microbial species play a large part in the biodeteriorative aspects of the disease. However, in order for microbes to gain access to and to colonise the internal structures of the hoof capsule, sites of entry must first be made available. This report looks at the role of nutritional factors and farriery regimens as the providers of such entry sites.

**Material and methods** A detailed questionnaire was formulated to cover the clinical lifestyle of 16 horses located within a 15-mile radius of Wigan in Lancashire. Factors pertinent to this report included in the survey were dietary intake, work load and type and farriery regimen. The equine lifestyles under investigation represented a divergent population. The appropriateness of diet with respect to work load was assessed using "Horsefed" software using 1989 N.R.C. guidelines. Hoof balance was assessed according to Rooney, (1980).

**Results** Of the 16 horses in the study, only 8 horses were shod all round, 50% of which were diagnosed with WLD. In contrast, there was no evidence of the condition in the 4 horses that were only shod at the front and of the remaining 4 unshod horses, only 1 animal presented signs of the disease.

**Table 1** The influence of farriery and dietary regimens on WLD prevalence.

		Proportion of Sample (%)				
		No WLD	WLD	No WLD	WLD	
<b>Anatomical Balance</b>				<b>Diet</b>		
Balanced	89	11		Appropriate	88	12
Unbalanced	43	57		Inappropriate	50	50
<b>Hoof Dressings</b>				<b>Dietary Suppl*</b>		
Applied	50	50		Fed	57	43
Not Applied	12	88		Not Fed	78	22

\*Dietary supplements including chondroitin sulphate, herbal preparations, biotin and vitamin preparations.

As shown in Table 1, only a small percentage (11%) of anatomically balanced equines exhibited signs of WLD, whereas 57% of their unbalanced counterparts were susceptible to the disease. In addition, equines whose hooves were frequently coated in hoof dressings showed a greater prevalence of WLD. Equines fed a diet deemed appropriate for their work load, had a lesser tendency to contract the disease than those fed an inappropriate diet. Furthermore, animals fed dietary supplements tended to exhibit a greater frequency of WLD than those whose diet was unsupplemented.

**Conclusions** The results presented herein provide direct evidence that farriery regimens and dietary management are important factors involved in the onset of WLD. In the case of farriery, unbalanced hooves leading to stress cracks and laminal separations provide ideal entry sites to the internal structures of the hoof capsule. The presence of nail holes within the capsule would provide alternative access routes. As with laminitis, the influence of diet on hoof horn quality should not be underestimated in WLD. Metabolic imbalance has dramatic effects on laminal nutrition and lamellar cohesion (Eustace, 1994), rendering the hoof capsule susceptible to microbial invasion.

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## Effect of three types of polysaccharide on fermentation parameters of a pony faecal inoculum when incubated *in vitro*

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**Introduction** Hind-gut acidosis in equines is associated with a number of debilitating and often fatal metabolic disorders such as colic and laminitis. It is well known that feeding equines large amounts of cereal starch can elicit the onset of hind-gut acidosis, but the role of other dietary polysaccharides in the aetiology of these disorders is less certain. It is believed that starch causes hind-gut acidosis through its preferential and rapid fermentation in the caecum by lactate-producing bacteria causing a decline in caecal pH. The aim of this experiment was to examine the effect of three types of polysaccharide on culture lactate and pH when incubated with a pony faecal inoculum. The polysaccharides used were cellulose, cereal starch and fructan, the storage carbohydrate of temperate grasses, which has been implicated, but not proven to be the causal agent of laminitis in pastured equines (Hinkley, 1997)

**Materials and Methods** Dried and ground (1mm) crystalline cellulose, fructan (as inulin) and maize starch (1 g per bottle, 27 replicate bottles per substrate) were each fermented *in vitro* with a faecal inoculum from a grass-fed pony, according to the method of Theodorou *et al.* (1994) for various periods up to 72 h. Cultures were serially sampled (3 bottles per substrate at each time point) 0, 4, 6, 8, 10, 12, 15, 20 and 72 hours post-inoculation, and measured for pH and lactate concentration.

**Results** Accumulation of lactate was significantly greater 8-12 h post-inoculation in the fructan cultures than the cellulose or starch cultures. The starch cultures accumulated more lactate than those containing cellulose 8 and 10 h post-inoculation whereas lactate concentrations in the cellulose cultures showed little change throughout the experiment (Table 1). Although all cultures showed an overall decline in pH with time, this was most pronounced for the fructan cultures which declined to pH 5.7 at 20h, and was significantly lower than those of the other cultures which never fell below pH 6.2 (Table 1). All substrates were almost completely fermented (>0.95) by the end of the experiment (data not tabulated).

**Table 1.** Time course of lactate (L) ( $\mu\text{g ml}^{-1}$ ) accumulation and pH change in batch cultures of fructan (as inulin), starch or cellulose fermented *in vitro* by a pony faecal inoculum

Substrate	Time post-inoculation (h)																	
	0		4		6		8		10		12		15		20		72	
	L	pH	L	pH	L	pH	L	pH	L	pH	L	pH	L	pH	L	pH	L	pH
Fructan	63	6.7	54	6.7	38	6.7	141 <sup>a</sup>	6.5 <sup>a</sup>	272 <sup>a</sup>	6.3 <sup>a</sup>	232 <sup>a</sup>	6.3 <sup>a</sup>	24	6.2 <sup>a</sup>	19	5.7 <sup>a</sup>	30	5.8 <sup>a</sup>
SE	5.2	0	2.8	0	1.4	0	2.8	0	20.2	0	9.9	0	0	0	0.4	0.1	1.9	0
Starch	76	6.7	43	6.7	32	6.7	59 <sup>b</sup>	6.5 <sup>a</sup>	42 <sup>b</sup>	6.4 <sup>b</sup>	25 <sup>b</sup>	6.4 <sup>b</sup>	22	6.4 <sup>b</sup>	20	6.5 <sup>b</sup>	22	6.2 <sup>b</sup>
SE	24	0	9.3	0	7.1	0	2.8	0	7.1	0	2.8	0	2.8	0	0.35	0.4	11.1	0
Cellulose	70	6.7	27	6.7	26	6.7	16 <sup>c</sup>	6.6 <sup>b</sup>	20 <sup>c</sup>	6.5 <sup>c</sup>	21 <sup>b</sup>	6.5 <sup>c</sup>	20	6.5 <sup>c</sup>	19	6.3 <sup>c</sup>	26	6.2 <sup>b</sup>
SE	2.2	0	3.0	0	11.3	0	1.9	0	2.8	0	3.5	0	4.8	0	20	0	12.9	0

<sup>a, b, c</sup> Values in columns with different superscripts are significantly different ( $P < 0.05$ )

**Conclusions** At their respective peaks of lactate accumulation, the fructan cultures accumulated approximately 4.5 and 10.5 times as much lactate as those containing starch or cellulose respectively and this was generally reflected in the lower pH of the fructan cultures, particularly later on in the fermentation. It is of note that it is generally accepted that a horse with a hind-gut pH of less than 6 is regarded as being acidotic. Despite the cultures being well buffered at the beginning of the experiment, the fructan culture pH fell well below this threshold of pH 6.

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# Influence of dietary protein supply on resistance to infection with *Haemonchus contortus* in Ile de France and Santa Ines lambs

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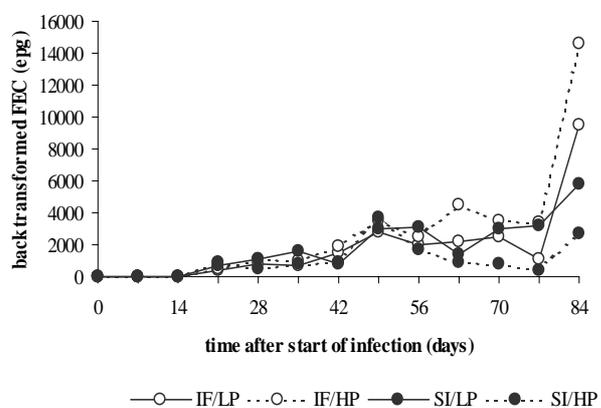
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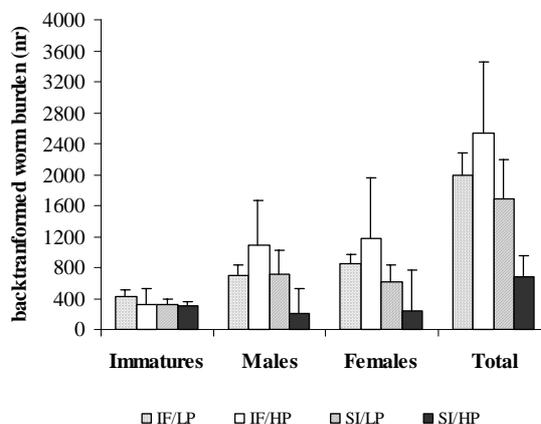
**Introduction** Anthelmintic resistance is rapidly increasing in the tropical and subtropical regions of Latin America, indicating the necessity for studies on alternative for the control of gastrointestinal nematodes in sheep. Protein supplementation has long been known to improve the resilience and resistance to gastrointestinal nematodes (Coop and Holmes, 1996). However, such effects of protein supplementation may differ between breeds of sheep. Santa Ines young sheep show higher resistance against natural infections with gastrointestinal nematodes than Ile de France and Suffolk sheep (Amarante, 2002). The purpose of this experiment was to determine whether a high level of soybean meal in the diet could improve the resistance to *Haemonchus contortus* in Ile de France and Santa Ines lambs.

**Material and Methods** Four-month-old male Ile de France (IF) or Santa Ines (SI) lambs, 12 of each breed, were randomly allocated to one of two iso-energetic foods, which were calculated to supply 75g (LP) or 129g (HP) metabolizable protein (MP) per kg dry matter (n=6). The diets consisted of low quality hay and pelleted concentrates at a 40:60 ratio. The pellets contained grass hay, ground maize, soybean meal and minerals. The lambs were fed *ad libitum*, allowing for 10-20% refusals. The lambs were trickle infected with 300 L<sub>3</sub> *H. contortus*, three times a week for 12 weeks. Body weight and faecal egg counts (FEC) were assessed weekly. The lambs were slaughtered for the assessment of worm burdens at the end of the 12-week experiment. FEC and worm burden data were log transformed prior to statistical analysis, and backtransformed means are presented. ANOVA was used to assess effects of breed, diet and their interaction, with FEC assessed through repeated measures (Proc GLM, SAS).

**Results.** Achieved feed intake averaged 1.3, 1.5, 1.3 and 1.4 kg fresh/day for the IF/LP, IF/HP, SI/LP and SI/HP lambs (SE 0.05). There was a significant breed × diet interaction on body weight gain, which averaged respectively 177, 258, 191 and 222 g/day (s.e.d. 13.9; P<0.05). Overall, FEC were not affected by diet but IF lambs tended to have higher FEC than SI lambs (Figure 1; P=0.093). However, time and treatments significantly interacted for FEC (P<0.01); a further analysis of the FEC revealed that the HP/SI lambs had lower FEC than the other groups on day 70 and 84 of the experiment (P<0.05). In support of this interaction, breed and diet also interacted for worm burdens; the SI/HP lambs had lower worm burden than SI/LP, IF/HP and IF/LP lambs (Figure 2; P<0.05).



**Figure 1.** Effect of breed and diet on FEC



**Figure 2.** Effect of breed and diet on worm burden (with 95% confidence interval)

**Conclusion** This experiment showed that an increased MP supply to SI lambs (resistant breed) increased resistance to *H. contortus*. In contrast, the increased MP supply did not improve the resistance of the Ile de France lambs (susceptible breed). The lambs that received increased MP supply showed a better performance regarding to weight gain, however, genetic resistance to nematodes may only be expressed at adequate level of protein nutrition. Overall, the study supports the view that improved protein supply to native breeds could enhance immunity to gastrointestinal nematodes and thus reduce the use of anthelmintics for parasite control.

**Acknowledgements** This study was supported by FAPESP (São Paulo - Brazil).

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## The effect of pre-weaning mixing and vitamin C supplementation on piglet performance

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**Introduction** It is well established that the weaning process is one of the most stressful periods for a piglet, which can alter its immune function and performance. Pigs have no specified dietary requirement for vitamin C with it being provided via colostrum and endogenous production. However, during stressful periods, physiological requirements increase, and supplementation may be required. The objective of this study was to investigate the effects of vitamin C supplementation in high and low stress weaning systems on performance and immune function of weaner pigs.

**Materials and Methods** A total of 24 PIC Camborough 15 (Large White x (Landrace x Duroc)) sows and their litters housed in farrowing crates, were randomly allocated to treatment in a 2 x 2 factorial design over three replicates. The treatments were mixed (M) or not mixed (NM) and vitamin C (VC) or no vitamin C (NVC). At 14 days of age, the boards between farrowing pens were removed allowing two litters to be mixed whilst the sows remained confined in their crates. Piglets were weaned at 28 days of age and moved into fully slatted weaner accommodation combining two litters together in one pen while remaining in their treatment groups. A feeding regime of four standard commercial diets was used across all treatments from day 7. The diet specification changed from 23% CP and 1.65% lysine pre-weaning to 21.5% CP and 1.4% lysine post weaning. Post weaning diets changed on day 35, 42 and 49 days of age. Vitamin C (Lutavit C Monophosphate® Frank Wright) was added to the basal diets at a rate of 0.3g/kg. Temperatures through the pelleting process were recorded and were within the products heat stable range. All piglets were weighed weekly from day 7 up until four weeks post weaning on day 56. At weaning two piglets from each litter were immunised intramuscularly with keyhole limpet haemocyanin (KLH) and class and sub-class anti-KLH antibody responses were assessed by ELISA. Statistical analysis was performed using ANOVA using Genstat version 6.

**Results** Creep feed intake during lactation was acceptable in all litters with an average of 388 g/pig from day 7 to day 28. There were significant main and interactive effects on intake pre weaning (Table.1). There was no significant mixing x vitamin C interaction on piglet daily live weight gain (DLWG) during the pre-weaning stage. There was a significant effect on DLWGs when piglets were supplemented with vitamin C during lactation (P<0.05 and P<0.01 respectively). Mixing piglets during lactation had no significant effect on DLWGs. There was no significant mixing x vitamin C interaction on piglets' feed intakes from day 7 to day 21. However, from day 21-28, piglets not mixed and receiving no vitamin C ate significantly more food (P<0.01). Piglets mixed pre-weaning and supplemented with vitamin C were significantly heavier on day 35 compared with all the other treatments (P<0.05). Piglets not mixed pre-weaning and receiving no vitamin C ate significantly more food three weeks following weaning (P<0.01). Piglets mixed prior to weaning and received vitamin C had a significantly greater anti-KLH IgG<sub>1</sub> response compared with mixed piglets and not receiving vitamin C on day 42 (P<0.05). However, piglets that were not mixed until weaning and receiving vitamin C had a significantly lower anti-KLH IgG<sub>1</sub> response compared with piglets mixed at weaning and receiving vitamin C on day 42 (P<0.05).

**Table 1. Effect of Mixing and Vitamin C on piglet performance and anti-KLH IgM response (OD<sub>405</sub>)**

		Mixed		Not Mixed		s.e.d	Mixing	Significance	
		VC	NVC	VC	NVC			VC	M*VC
Pre-weaning VFI	g/d	16.02	16.40	15.32	26.15	2.106	0.007	0.001	0.002
Post-weaning VFI	g/d	404.4	470.3	427.9	558.3	30.02	0.039	0.004	N.S
*Day 7-14	g/d	297	262	283	252	20.2	N.S	0.038	N.S
*Day 14-21	g/d	309	264	312	266	19.2	N.S	0.003	N.S
*Day 21-28	g/d	310	248	304	297	22.9	N.S	0.039	N.S
*Day 28-35	g/d	86	68	35	142	30.7	N.S	N.S	0.009
*Pre-weaning DLWG	g/d	290	246	286	259	16.9	N.S	0.008	N.S
*Day 28 live weight	kg	9.27	8.13	9.05	8.49	0.382	N.S	0.005	N.S
*Day 35 live weight	kg	9.84	8.70	9.43	9.53	0.352	N.S	0.045	0.020
*Post-weaning DLWG	g/d	586	574	580	638	34.6	N.S	N.S	N.S
# Anti-KLH IgG <sub>1</sub> d 42		1.204	1.046	1.009	1.173	0.0975	N.S	N.S	0.036
# d 49		1.045	1.009	1.035	1.001	0.1178	N.S	N.S	N.S

\* Adjusted means using day 7 weight as a covariate; # Adjusted means using day 28 OD<sub>405</sub> as a covariate

**Conclusion** Beneficial effects on piglet live weights can be gained by supplementing the diet with vitamin C from 7 days of age up until the first week after weaning. In addition to vitamin C supplementation, mixing piglets at 14 days of age can improve piglet weaning weights. Supplementing the diet after one week post-weaning had little effect on piglet performance. However, the interaction between pre-weaning mixing and vitamin C supplementation on the piglets' immune response requires further investigation to look at other components of the immune system.

## Ontogeny of factors thought to control the development of ovine muscle in utero

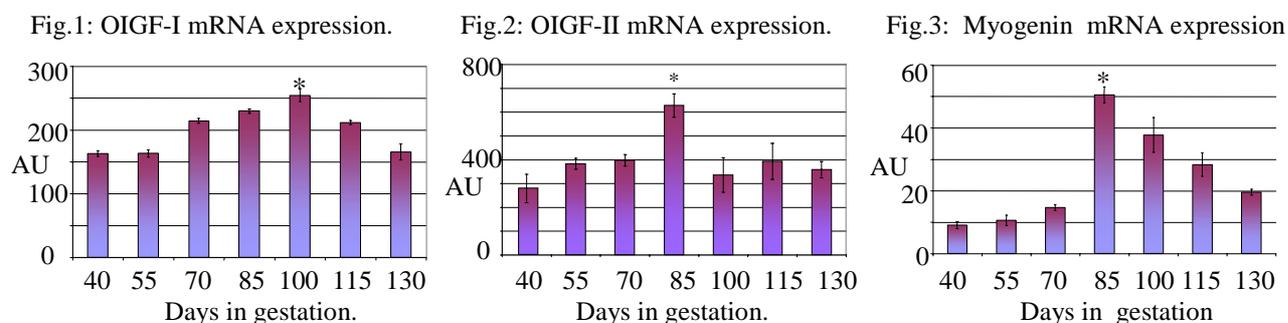
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**Introduction** It is believed that there are time periods during the lifetime of an animal when inadequate nutrition may result in the animal not reaching its genetic potential. One sensitive period is during early foetal life at the time when muscle fibres are being formed from myogenic cells. The total numbers of muscle fibres for life are essentially set during this period. The aim of this study was to try and identify the ontogeny of muscle cell differentiation by quantifying the mRNA expression of ovine IGF-I, ovine IGF-II, and ovine myogenin, factors known to influence myoblast proliferation or differentiation. The aim was to gain an indication of the time period during gestation at which differentiation of the muscle cells commences. The period before differentiation (i.e. during proliferation) is potentially sensitive to external factors, such as nutrient supply. This information could be used to determine the time during pregnancy when adequate feeding is essential to ensure maximum muscle development of the offspring, and may allow the manipulation of diet to change muscle characteristics for meat production.

**Materials and methods** Twenty-three pregnant ewes (North Country mules) carrying twins were used in this study. The ewes were mated naturally and checked every 2 days so that the raddle mark could be observed and recorded. Day zero of gestation was taken as the first day at which the ewes were observed to have an obvious raddle mark. On day 40 the ewes (n=3) were weighed and then injected with an overdose of pentobarbitone sodium Ph.Eur (200ng/ml) (recommended dose 1ml/1.5kg body weight). The ewes were cut open and the uterus removed intact. The foetuses were located and removed and the hind limb muscle dissected and snap frozen in liquid nitrogen. This same procedure was carried out approximately every 15 days, until term. At each time period the number of ewes = 3 except at day 55 and day 70 n=4. The older foetuses (day 70 +) were also administered an overdose of sodium pentobarbitone via cardiac puncture. In very early gestation it would have been impossible to dissect individual hind limb muscles, therefore the muscle sample taken was of mixed hind limb muscles. The mRNA expression of ovine IGF-I, ovine IGF-II was measured using ribonuclease protection assays, as a unit of total RNA. The mRNA expression of ovine myogenin was measured relative to the control (18s) using quantitative reverse transcriptase PCR (Taqman, ABI Biosystems). The data was analysed by ANOVA, using the Tukey test as a post hoc analysis.

**Results** The expression of ovine IGF-I (OIGF-I) mRNA in the foetal muscle samples peaked at 100 days, with higher levels at d70, d85 and d100 compared with d115 and 130 ( $p < 0.0001$ ) (figure 1) (\* = d100 compared to d130  $p < 0.005$ ) The expression of ovine IGF-II (OIGF-II) mRNA in the foetal muscle samples peaked at 85 days gestation ( $p < 0.005$ ) (figure 2), (\* = d85 compared to d40-d100, and d130  $p < 0.05$ ) as does the expression of ovine myogenin mRNA ( $p < 0.0001$ ) (figure 3) (\* = d85 compared to d40-d70, d115-130  $p < 0.0001$  and d85 compared to d100  $p < 0.05$ ).



**Conclusions** We have previously shown that there are high levels of IGF-II mRNA expression in proliferating foetal sheep myoblasts with a decline in IGF-II mRNA with increased differentiation, therefore the peak at 85days could indicate the end of myoblast proliferation. (Brameld *et al* 1998). Studies observing OIGF-I expression have shown higher levels of total IGF-I mRNA in foetal sheep muscle at d84 compared to d134 (Dickson *et al* 1991). A decrease in IGF-I mRNA is thought to occur once muscle cells are fully differentiated and innervated. The appearance of myogenin probably coincides with the onset of differentiation. IGFs have been shown to increase myogenin *in vitro*. Myogenin has been shown to be an early marker of the onset of differentiation, since the lack of myogenin leads to perinatal death in newborn mice due to severe deficiency of differentiated muscle fibres. (Hasty *et al* 1993). The data presented suggests that muscle proliferation occurs before 85days of gestation and that differentiation commences around day 85.

**Acknowledgements** A.J.Fahey was supported by a BBSRC studentship.

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# Intake, liveweight gain and feed conversion in organic Scottish-Blackface lambs finished on contrasting clover based silages and concentrates with different ratios of wheat and beans

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**Introduction** Clover based silages offer an opportunity for both conventional and organic upland producers to add value and improve the marketability pattern of organic hill and upland lambs. The objective of this study was to examine the performance of weaned hill lambs finished on contrasting clover based silages (SIL) supplemented with organic concentrates (CONC) containing varying ratios of wheat and beans.

**Materials and methods** A 2 x 4 factorial continuous design experiment was conducted to determine voluntary dry matter intake (DMI), liveweight gain (LWG), feed conversion ratio (FCR) and carcass characteristics in weaned Scottish-Blackface male lambs. Two 2<sup>nd</sup> cut silages were made in round bales from either a perennial ryegrass/white clover sward (PRG/WC) or a pure red clover sward (RC<sub>2</sub>). The 4 CONC treatments comprised rolled wheat and crushed beans in the ratios of 100:0 (W), 67:33 (WB), 33:67 (BW) and 0:100 (B), molasses and minerals. CONC were fed to all lambs at the rate of 450 g/head/day in two equal meals and silages were chopped and available *ad libitum*. A 1<sup>st</sup> cut RC silage (RC<sub>1</sub>) was fed during the last 3 weeks of the trial instead of the 2<sup>nd</sup> cut material. Silage composition for the PRG/WC, RC<sub>1</sub> and RC<sub>2</sub> was as follows: (DM) 229, 592, 233 (g/kg); (ME) 12.0, 8.7, 8.1 (MJ/kg DM); (CP) 210, 140, 215 (g/kg DM). CONC DM and ME were similar at 838 g/kg and 13.2 MJ/kg DM whilst CP levels were 136, 196, 256 and 296 g/kg DM for the W, WB, BW and B CONC's respectively. In total, 144 male lambs from organically managed flocks at ADAS-Redesdale were used. Lambs were housed in 24 straw-bedded pens (6 lambs/pen) giving 3 replicate pens for the 8 (2x4) treatment combinations. DMI was determined for each pen over 4 days in weeks 3 and 6 of the trial and individual lamb LWG determined by linear regression on weekly liveweight (LW) data. Cold carcass weight (CCW), killing out proportion (KO) and fatness and conformation carcass gradings were obtained for each lamb (target slaughter condition of R3L). Analysis of variance for DMI and FCR (assuming average LWG in weeks 3 and 6) were carried out on a pen basis and for LWG and carcass data on an individual lamb basis using Genstat 5.

**Results** Concentrate (CDMI), silage (SDMI) and total (TDMI) intake figures, LWG, FCR (g DMI/g LWG) and slaughter characteristics for the main SIL and CONC effects are given in Table 1. No significant differences were seen in LWG, days on trial, KO or fat or conformation gradings between treatment factors. SDMI was significantly higher (P<0.001) for lambs given RC compared with PRG/WC silage in both weeks and for lambs given B compared with W, WB or BW CONC (P<0.05) in week 3 only. Forage:concentrate ratio averaged 604 and 542 g/kg TDMI for the RC and PRG/WC diets respectively so lambs were not sold on an organic basis. Feeding beans (B and WB in week 3 and B in week 6) in the CONC supplement significantly increased FCR (P<0.05) compared to feeding W alone. Feeding RC silage increased slaughter LW by 2 kg (P<0.001) and CCW by 0.6 kg (P<0.05) compared to feeding PRG/WC silage. Gross Margins (£/lamb) did not differ across treatment factors and averaged £4.69 on a conventional sale/feed pricing basis and £6.69 assuming lambs could have been sold using published organic price schedules and organic feed prices.

**Table 1** Intakes, LWG, FCR and carcass characteristics in finishing Scottish-Blackface male lambs.

Year		Silage			Concentrates				sed	Sig of effects	
		PRG/WC	RC	sed	W	WB	BW	B		SIL	CONC
Week 3	CDMI (g/d)	383	383		388	386	382	378			
	SDMI “	<u>486</u>	<u>582</u>	22.9	<u>508<sup>a</sup></u>	<u>522<sup>a</sup></u>	<u>505<sup>a</sup></u>	<u>598<sup>b</sup></u>	32.4	***	*
	TDMI “	<u>869</u>	<u>964</u>	22.9	<u>896<sup>a</sup></u>	<u>908<sup>a</sup></u>	<u>888<sup>a</sup></u>	<u>976<sup>b</sup></u>	32.4	***	*
	g/kg LW <sup>0.75</sup>	66.9	73.6	1.70	68.3	69.5	68.6	74.6	2.40	**	
Week 6	CDMI (g/d)	380	380		383	383	378	376			
	SDMI “	<u>430</u>	<u>592</u>	12.9	<u>524</u>	<u>495</u>	<u>497</u>	<u>528</u>	18.2	***	
	TDMI “	<u>810</u>	<u>972</u>	12.9	<u>907</u>	<u>878</u>	<u>875</u>	<u>904</u>	18.2	***	
	g/kg LW <sup>0.75</sup>	60.9	72.0	0.88	67.3	65.2	65.7	67.5	1.24	***	
LWG (g/day)	72	80	5.2	85	72	75	70	7.3			
FCR week-3	12.7	12.5	0.62	10.7 <sup>a</sup>	12.9 <sup>b</sup>	12.1 <sup>ab</sup>	14.7 <sup>b</sup>	0.88		*	
FCR week-6	11.9	12.5	0.62	10.8 <sup>a</sup>	12.5 <sup>ab</sup>	11.8 <sup>ab</sup>	13.6 <sup>b</sup>	0.87		*	
Days on trial	84	94	5.3	84	88	85	97	7.5			
Slaughter LW (kg)	35.3	37.3	0.54	36.5	36.2	35.6	36.8	0.77	***		
CCW (kg)	14.9	15.5	0.26	15.2	15.1	15.3	15.3	0.37	*		
KO (g/kg)	424	416	0.49	416	418	430	416	0.69			

For concentrates, values not sharing common superscripts differ significantly (P<0.05).

**Conclusions** Although both silages were suitable for finishing hill lambs, RC silage was consumed in greater quantities than PRG/WC silage. However LWG did not increase with RC silage, perhaps due to its lower estimated ME content. Increasing the CP content of the CONC by including beans was not justified since it did not improve lamb performance.

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

# Liveweight and pre-weaning growth in suckled calves sired by either Aberdeen Angus or Charolais bulls from contrasting autumn-calving continental x dairy cows over three years

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**Introduction** Little information is available on the performance of suckled calves sired by either traditional UK beef breeds or continental beef breeds in conjunction with modern continental x dairy suckler cow genotypes. The objectives of this study were to determine gestation length, calf liveweight (LW) and pre-weaning daily liveweight gain (DLWG) from contrasting suckler dam and terminal sire breeds when managed within an upland suckler herd.

**Materials and methods** Two autumn-calving sub-herds of either 50-60 Belgian Blue x Holstein (BB) or 50-60 Simmental x Holstein (SIM) animals considered typical of either a beef/carcass type or a milky/maternal type of continental x dairy suckler cow were maintained at ADAS-Redesdale. In each year, approximately half the cows in each sub-herd were sired with Aberdeen Angus (AA) or Charolais (CH) sires except for replacement heifers that were always sired by AA sires. Five high beef-value AA sires and five high beef-value CH sires were chosen for use with artificial insemination (AI) over a 6-week mating period followed by a further 6-week mating period when a “sweeper” bull of the appropriate sire breed was used. As well as *ad libitum* access to grass hay, calves received a home-mixed barley/soyabean meal concentrate at an average of 1.5 kg/head/day from 120 days of age until weaning. Suckled calf LW was determined at regular intervals from birth until weaning at 8 months old and gestation length (GL) from mating and calving records. Calf birth weights (BW), 100 day weights (100DW), 200 day weights (200DW), weaning weights (WEAN), DLWG and GL were recorded during each of the years between 1999-2002 (99-00, 00-01 and 01-02). Data for each year were analysed as an unbalanced 2 x 2 x 2 factorial (dam breed x sire breed x calf sex) using the residual maximum likelihood (REML) facility in Genstat 5.

**Results** Dam (D) by sire (S) interaction average values and the significance of the D, S, Sex and their main interactions for calf BW, 100DW, 200DW, WEAN, DLWG and GL in each year are given in Table 1. For all variables except WEAN in 01-02 and GL in 99-00, CH sired calves were associated with higher values (P<0.05) than AA sired calves and for all variables except BW and GL in 99-00, male calves were associated with significantly (P<0.05) higher values than female calves (values not shown). SIM cows only produced significantly heavier (P<0.05) calves at 200 days in 00-01 and calves with higher DLWG (P<0.05) in 01-02 compared with BB cows. For some variables, significant interactions (P<0.05) were seen between DxS across all years and between DxSxSex for all variables in all years.

**Table 1.** Liveweights, liveweight gain and gestation length in suckled calves pre-weaning.

	Year	Breed combination				sed	Dam	Significance of effects			
		AA/BB	AA/SIM	CH/BB	CH/SIM			Sire	Sex <sup>\$</sup>	DxS	DxSxSex
BW (kg)	99-00	41 <sup>a</sup>	41 <sup>a</sup>	49 <sup>b</sup>	48 <sup>b</sup>	1.6		***			*
	00-01	45 <sup>a</sup>	45 <sup>a</sup>	52 <sup>b</sup>	53 <sup>b</sup>	1.9		***	**		*
	01-02	45 <sup>a</sup>	44 <sup>a</sup>	50 <sup>b</sup>	48 <sup>ab</sup>	1.8		***	**	*	*
100DW (kg)	99-00	141 <sup>a</sup>	143 <sup>a</sup>	157 <sup>b</sup>	163 <sup>b</sup>	4.6		***	*		*
	00-01	154 <sup>a</sup>	158 <sup>ab</sup>	165 <sup>bc</sup>	172 <sup>c</sup>	4.9		***	***	*	*
	01-02	149 <sup>a</sup>	152 <sup>a</sup>	163 <sup>b</sup>	164 <sup>b</sup>	4.2		***	*		*
200DW (kg)	99-00	259 <sup>a</sup>	263 <sup>a</sup>	285 <sup>b</sup>	293 <sup>b</sup>	7.7		***	**		*
	00-01	263 <sup>a</sup>	273 <sup>ab</sup>	282 <sup>bc</sup>	295 <sup>c</sup>	8.2	*	***	***	*	*
	01-02	253 <sup>a</sup>	263 <sup>a</sup>	278 <sup>b</sup>	282 <sup>b</sup>	6.6		***	**		*
WEAN (kg)	99-00	296 <sup>a</sup>	304 <sup>ab</sup>	320 <sup>bc</sup>	335 <sup>c</sup>	8.3		***	***	*	*
	00-01	315 <sup>a</sup>	313 <sup>a</sup>	322 <sup>ab</sup>	341 <sup>b</sup>	11.5		*	***	*	*
	01-02	298	307	310	315	9.6			*		*
DLWG (kg/d)	99-00	1.10 <sup>a</sup>	1.11 <sup>a</sup>	1.18 <sup>b</sup>	1.24 <sup>b</sup>	0.031		***	*		*
	00-01	1.10 <sup>a</sup>	1.12 <sup>ab</sup>	1.12 <sup>ab</sup>	1.21 <sup>b</sup>	0.034		**	***	*	*
	01-02	1.04 <sup>a</sup>	1.11 <sup>b</sup>	1.15 <sup>bc</sup>	1.18 <sup>c</sup>	0.030	*	***	**	*	*
GL (Days)	99-00	286	287	289	288	1.9					*
	00-01	283 <sup>a</sup>	284 <sup>ab</sup>	287 <sup>ab</sup>	289 <sup>b</sup>	1.7		*	*	*	*
	01-02	284 <sup>a</sup>	286 <sup>ab</sup>	288 <sup>b</sup>	288 <sup>b</sup>	1.8		*	*	*	*

Values not sharing common superscripts differ significantly (P<0.05). \$. Male calves left entire in 99-00 and 00-01.

**Conclusions** Generally, CH calves were heavier and grew faster than AA calves and male calves were heavier and grew faster than female calves. Dam type had little effect on calf performance except in two cases where calves from SIM cows out-performed calves from BB cows. Interactions indicated that CH calves from SIM cows out-performed AA calves from BB cows. GL was higher for CH than for AA sired calves and for male compared with female calves.

**Acknowledgements** This work was funded by DEFRA, MLC, Dovecote Park and Waitrose Ltd with further support from the Aberdeen Angus Cattle Society and the British Belgian Blue Cattle Society.

## Evaluation of an ensiled mix of moist sugar beet feed (pressed pulp) plus maize distillers dark grains as a supplement for twin-bearing March-lambing ewes fed straw-based diets

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**Introduction** Feeding lowland sheep on straw-based systems during pregnancy is practised on many livestock/arable farms. Simple mixes of molassed sugar beet feed and distillers dark grains have been cost effective supplements for March-lambing ewes fed straw and produced satisfactory ewe and lamb performance (Chapple *et al.*, 1998 and 2001). An ensiled mix of pressed sugar beet pulp and dried maize distillers grains (Praize, Trident Feeds) has been fed as the sole diet for finishing lambs (Pattinson *et al.*, 2001) but there is little information on feeding Praize to pregnant ewes. The objective of this study was to compare ewe and lamb performance when March-lambing ewes were fed on a straw-based system and supplemented with either a cereal/protein home-mix, Praize or one of two dried sugar beet pulp/protein mixes.

**Materials and Methods** 120 March-lambing Mule and Suffolk cross ewes, scanned as carrying twins, were housed and divided into four treatment groups (two pens of 15 ewes/treatment) on 22 January 2002. All ewes were fed *ad libitum* barley straw supplemented with either rolled wheat/rapeseed meal 70:30 (WR), maize distillers/molassed sugar beet feed 60:40 (MB), molassed sugar beet feed/soya bean meal 78:22 (BS) or ensiled pressed sugar beet pulp/dried maize distillers 80:20 (PBM). Supplementary feeding started seven weeks prior to lambing at 0.60 kg/head and gradually increased up to 1.15 kg/head at lambing for WR (ME 12.7 MJ/kg, CP 208g/kg), MB (ME 13.0 MJ/kg, CP 228g/kg) and BS (ME 12.4 MJ/kg, CP 198g/kg). PBM (ME 12.3 MJ/kg, CP 190g/kg) was fed on the same dry matter basis as the other rations. After lambing, ewes and lambs were turned out to perennial ryegrass/white clover swards where they were supplemented with 0.60 kg/head of molassed sugar beet feed. Lambs did not receive any creep feed and the experiment finished when they were approximately seven weeks old. Ewe and lamb live weights, ewe body condition score and feed intakes were recorded. The experiment was a randomised block design and data were analysed using analysis of variance.

**Results** All ewes lost weight up to lambing and regained some weight after lambing. There were no significant differences between treatments (Table 1). Ewes on all treatments lost some body condition (0.8 condition score) up to lambing but increased to body condition score 2.8 by the end of the experiment.

**Table 1** Ewe Performance

	WR	MB	BS	PBM	s.e.d.
Live weight (kg) :					
Start weight (21 Jan)	78.2	78.5	78.3	78.2	0.44
Lambing weight (24 March)	71.9	69.7	72.3	70.1	1.23
Final weight (9 May)	73.5	75.2	74.8	73.7	1.60
DM intake (kg/day)	1.73	1.81	1.82	1.75	0.074
ME intake at lambing (MJ/day)	19.5	19.6	20.7	17.8	1.58

Lamb birth weights, seven-week weights and growth rates were similar for all treatments (Table 2).

**Table 2** Lamb Performance

	WR	MB	BS	PBM	s.e.d.
Birth weight (kg)	5.15	4.96	5.18	5.20	0.157
7-week weight (kg)	19.6	20.3	20.1	20.3	0.61
Daily gain: Birth-7 weeks (g)	324	333	332	327	9.3

**Conclusion** An ensiled mix of moist sugar beet pulp and maize distillers dark grains can replace more conventional dry feed supplements of rolled wheat/rapeseed meal, maize distillers/molassed sugar beet feed or molassed sugar beet feed/soya bean meal when fed to twin-bearing ewes on straw diets without affecting ewe or lamb performance.

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# Extensive finishing of weaned suckled heifers sired by Aberdeen-Angus or Charolais bulls from autumn-calving continental cross cows

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**Introduction** Suckled calf production is the only financially viable cattle enterprise for the hills and uplands. The use of continental cross cows derived from the dairy herd has increased dramatically, in pursuit of faster-growing, higher-value progeny, and as a strategy to compensate for the adverse effect of the Holstein on carcass conformation. Continental sires are normally used on these types of suckler cows but the traditional Aberdeen-Angus sire is now selling at a premium and might be better suited for upland production. Previous ADAS trials have shown the potential of continental cross cows to increase calf growth rates in a hill environment (Keatinge *et al.*, 1994), and to improve carcass quality when the progeny are finished intensively (Chapple *et al.*, 1995). The objective of this experiment was to evaluate the performance of weaned heifers finished extensively at 22-24 months of age.

**Materials and methods** Aberdeen-Angus (AA) or Charolais (CH) sires were used on either Simmental x Holstein (SM) or Belgian Blue x Holstein (BB) suckler cows. Forty autumn-born heifer calves (10 of each breed type) were weaned at approximately eight months of age. They were turned out, in May (mean live weight 323 kg), to graze on permanent pasture swards at high stocking rates to achieve low growth rates at grass. The cattle were housed in October and fed grass silage during the winter and then finished on high quality perennial ryegrass/white clover swards at 22-24 months of age. The heifers were selected for slaughter by subjective handling, when judged to be at European Union (EU) external fatness score 4L. At slaughter, individual joints were weighed and the proportion of saleable meat, bone and fat trim were determined. All the data were analysed by analysis of variance.

**Results** Progeny sired by CH were always significantly heavier ( $P<0.01$ ) than progeny sired by AA. Growth rates were higher for AA heifers in yards and for CH heifers at grass in the second summer. Overall growth rates were similar for both sire types. However, progeny sired by CH produced significantly heavier carcasses ( $P<0.001$ ), had higher dressing proportions ( $P<0.01$ ), higher yields of saleable meat ( $P<0.01$ ) and a lower proportion of fat trim ( $P<0.001$ ) than progeny sired by AA. There was no significant effect of dam type. A premium of 28p/kg was paid for AA sired cattle and although carcass weights were approximately 19 kg lighter than CH sired heifers the AA cattle realised an extra £48/head (£561 cv. £513).

**Table 1** Finishing Performance by dam and sire type (10 heifers per treatment).

Dam Sire	SM	BB	SM	BB	s.e.d.	Significance	
	AA	AA	CH	CH		Dam	Sire
Sale weight (kg)	547	532	558	566	10.0	NS	**
Growth rates (kg/day):							
At grass (142 days)	0.29	0.25	0.29	0.27	0.041	NS	NS
In yards (182 days)	0.51	0.39	0.32	0.37	0.048	NS	**
Second summer	0.77	0.79	1.02	0.96	0.060	NS	***
Overall	0.51	0.47	0.50	0.51	0.022	NS	NS
Carcass wt (g/kg)	289	279	300	305	6.1	NS	***
Dressing (g/kg)	529	525	536	539	5.6	NS	**
Carcass composition:							
Saleable meat (g/kg)	704	697	715	721	8.1	NS	**
Bone (g/kg)	222	232	235	230	6.9	NS	NS
Fat trim (g/kg)	74	71	50	49	5.1	NS	***

**Conclusions** High quality animals were produced from both sire types. CH sires produced heavier carcasses, had higher dressing proportions, higher yields of saleable meat and less fat trim than AA sires. However, these results illustrate that with a premium for AA heifers a financial advantage (£48/head) can be achieved when suckled heifers are finished on an extensive system.

**Acknowledgements** This research was funded by DEFRA, MLC, Waitrose, Dovecote Park and Breed Societies.

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# Improvement of the growth and performance of Holstein neonatal calves receiving the microbial additive *Saccharomyces cerevisiae*

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**Introduction** Yeasts such as strains of *Saccharomyces cerevisiae* (SC) are now widely used as additives in ruminant nutrition to improve animal performance, health and utilization of nutritional components of their diet while at the same time avoiding nutritional disorders. Although evidence for a positive effect on animal performance has existed for many years, adoption has been slow. This is probably due to the lack of performance data indicating in which specific production situations the various products are or are not effective. Some studies indicated that the feeding of a yeast strain specifically selected to compliment high grain diets (Yea-Sacc, Alltech, Inc., USA) was effective in improving gain of yearling steers fed a 90% concentrate, 10% roughage diet once daily. So the objective of this study was to determine if this Bakery's yeast product could positively affect Holstein neonatal calves growth and performance.

**Materials and methods** Eighteen female Holstein neonatal calves were used in this study. They were randomly placed on treatments in a completely randomized design and fed colostrums at 10% of birth weight and milked until 45 days old. All calves were fed calf starter (NRC 2001) containing high quality alfalfa (15%) from seven days of age and weaned at 45 days. Calf starter was offered until 90 days old and the yeast was added at 0, 0.5 and 1 percent to the calf starter, which was, used daily. The weight, frame measures and rectal temperature of calves were taken from 0 to 90 days in regular periods (15days). Feed intake was measured daily.

**Results** As can be seen in table 1, there is a difference between daily dry matter intake (DMI) of calves ( $P \leq 0.0001$ ) and rectal temperature ( $P \leq 0.05$ ) in treatments but no differ in average daily gain (ADG), calves weight in periods, metabolic weight, feed efficiency and rumen pH. Seymour *et al.* (1995), also indicate that inclusion of yeast in a complete calf ration reduced the incidence of elevated body temperature and antibiotic treatment in young calves from birth to weaning, but had no effect on growth rate or efficiency and increased the DMI. Addition of SC to the calf starter had no effect on body length, pin width, hip width, pin to hook length, metacarpus and metatarsus size but had a considerable effect on wither height ( $P \leq 0.009$ ), hip height ( $p \leq 0.029$ ), stomach size ( $p \leq 0.002$ ) and hearth girth ( $p \leq 0.015$ ) in treatments. Chaucheyras *et al.* (2001) suggest that the higher level of cellulolytic bacteria in the rumen of lambs receiving the yeast additive indicate that the yeasts may enhance the development of ruminal functions and also the ability of live SC to consume oxygen entering the rumen could affect, rapid absorption of fermentation gases and oxygen from rumen and reducing its volume so cause the decrease in stomach size.

**Table 1** Means of some measures during the experiment

Item	0% yeast (T1)	0.5% yeast (T2)	1% yeast (T3)	SEM
Daily DMI (kg)	1.05 <sup>a</sup>	0.93 <sup>b</sup>	0.93 <sup>b</sup>	0.37
Rectal temperature	39.36 <sup>a</sup>	39.19 <sup>b</sup>	39.35 <sup>a</sup>	0.43
ADG (kg)	0.38 <sup>a</sup>	0.35 <sup>a</sup>	0.36 <sup>a</sup>	0.19
Calves weight	57.97 <sup>a</sup>	55.99 <sup>a</sup>	56.65 <sup>a</sup>	7.92
Metabolic weight	20.84 <sup>a</sup>	20.35 <sup>a</sup>	20.53 <sup>a</sup>	2.09
Feed efficiency	1.01 <sup>a</sup>	0.98 <sup>a</sup>	0.95 <sup>a</sup>	0.29
Rumen pH	5.79 <sup>a</sup>	5.78 <sup>a</sup>	5.94 <sup>a</sup>	0.46
		<u>Frame measurements</u>		
Wither height	84.06 <sup>a</sup>	82.67 <sup>ab</sup>	81.69 <sup>b</sup>	3.22
Hip height	89 <sup>a</sup>	88.22 <sup>ab</sup>	86.89 <sup>b</sup>	3.34
Stomach size	106.70 <sup>a</sup>	102.60 <sup>b</sup>	105.13 <sup>a</sup>	4.77
Hearth girth	92.93 <sup>a</sup>	91.03 <sup>b</sup>	91.71 <sup>ab</sup>	2.76

**Conclusions** The results of this study demonstrate that increase in the amount of SC can reduce DMI without significant effect on the ADG, weight and feed efficiency of calves, metabolic weight and rumen pH (Increased numerically) and can reduce wither height and hip height. As can be seen in table 1, use of 0.5% SC could significantly reduced the rectal temperature of calves and had significant effect on the stomach size and hearth girth in compare with 0% yeast (T1).

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# Use of visual image analysis for the management of pig growth in size and shape

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**Introduction** The purpose of an integrated management system is to optimise both pig performance and environmental protection. A major impediment to this process has been the inability to measure and control the production process in real time, for specific and contemporary pig batches. Optimisation thus requires in-line measurement of pig growth performance, together with the means to change performance with adjustments to feed quantity and quality. This report deals with the use of visual image analysis (VIA) to provide the first of these; measurement of growth. VIA determines, continuously and in-line, the size and shape of the plan view of the pig as it stands at the feeder. Three seminal questions are here addressed. (i) can VIA be used to provide a reliable measure of pig weight, and (ii) how many days are required to elapse before a change in size can be reliably determined and how does the VIA system compare with daily weighing by a conventional weigh-scale, and (iii) can VIA sort pigs according to their shape?

**Materials and methods** Forty pigs each of three commercial pig types (50% Landrace, 50% Pietrain, 25% Meishan) from Large White × Landrace mothers were placed onto trial as part of a serial slaughter experiment from 25 to 115 kg live weight. The pigs were fed *ad libitum*. Visual imaging and live weight data were collected daily from an electronic feeding station (FIRE Feeder, Osborne Europe, Ltd) adapted to carry the visual imaging hardware above it. An ANCOVA model determined the relationship between live weight and pig plan area for all data, to test for type effects.

**Results** The model of live weight (kg) on plan area of the pig ( $A_4$ , cm<sup>2</sup>) showed a robust relationship ( $P < 0.001$ ), and that there were significant differences between the pig types in constant but not slope ( $P < 0.05$ ). Differences are presumed due to type differences in shape and conformation.

“Landrace type”                      Live weight =  $0.049(0.0005) \times A_4 - 29.8(0.74)$

“Pietrain type”                      Live weight =  $0.052(0.0004) \times A_4 - 38.5(0.67)$

“Meishan type”                      Live weight =  $0.052(0.0004) \times A_4 - 32.8(0.63)$

The model had an  $r^2$  of 0.905 and the residual standard deviation was 6.22 kg.

The pigs grew daily an average of 14.9 cm<sup>2</sup>  $A_4$  and 0.829 kg live weight. The number of days required to find with 95% confidence that a pig has changed its size or weight is given by determination of the point at which the accumulated increase in weight divided by the standard error of the difference is greater than 1.65. For the “Landrace”, “Pietrain” and “Meishan” types, the number of days so computed were: for conventional weighing 10, 11 and 12 days, and for VIA  $A_4$  measurement 13, 11 and 14 days.

A radial basis function neural network was employed to determine if the three pig types could be sorted into groups according to their visually imaged characteristics of size and shape. A subset of the data was used for training. Effectiveness of true sorting according to type (excluding the training dataset) was 64% for the “Landrace” type, 81% for “Pietrain” and 81% for “Meishan”.

**Conclusions** The general relationship between VIA measurement and live weight is good, with differences between pig types consistent with a proposition that VIA can distinguish between pigs of differing plan area at any given weight. Given the equations presented VIA is a realistic alternative to the weigh scale to monitor pig performance. Visual imaging required two more days (13 vs 11) than daily weighing to detect a change in size or weight with a high degree of confidence. The interval for VIA is considered acceptable for real-time growth control within an integrated management system, and it is suggested that VIA may be operationally easier to manage than conventional weighing. The apparent ability of visual imaging to sort the three commercial pig types into their appropriate groups may be taken to suggest that the technique may have much to offer with regard to live sorting according to pig shape and conformation. This option is, of course, not available with conventional weigh scales. The relationship between visual image and carcass value is presently under further exploration.

**Acknowledgements** This work is part of the UK DEFRA LINK programme *Integrated Management Systems for Pig Nutrition Control and Pollution Reduction*. The authors acknowledge the support of DEFRA, MLC, BOCM Pauls Ltd, PIC (UK) Ltd, Osborne (Europe) Ltd.

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## Too close for comfort? The effects of social facilitation on feeding behaviour in the horse

### (*Equus caballus*)

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**Introduction** Social facilitation has been observed in the stabled horse with access to forage (Sweeting et al. 1985). Socially facilitated feeding behaviour has not been investigated through the provision of concentrates. It is likely that the motivation to ingest a concentrate feed is different to that of forage. In a variety of species social facilitation will only occur when presented with a novel food. Therefore it has been proposed that a function of social facilitation is to increase the acceptance of novel feeds. The first objective of this study therefore, was to investigate if social facilitation occurs with the horse's standard concentrate feed and or a standard concentrate feed plus a novel flavour.

Visual contact has been found to be a necessary component in the facilitation of forage ingestion. Increasing visual contact between stables improves awareness of conspecifics, which has been linked to a decrease in abnormal behaviour. There has, however, been limited investigation into the relationship between neighbouring stabled horses especially during potentially stressful periods such as meal times. The second objective of this study was to investigate the effects on behaviour of the presence or absence of a familiar horse during meal times.

**Materials and methods** Seven riding horses of mixed breed, six geldings and one mare, age range 8-21 years, were used. Six acted as observers and one horse, a gelding, was chosen to be a demonstrator due to a quiet temperament and motivation to feed; all horses were familiar with each other. Following a period of adjustment to the trial arena and equipment two trials were carried out. The first trial used a standardised plain feed (200g) and the second trial used the same feed (400g) plus one of each of three flavours (red fruit, aniseed and thyme<sup>†</sup>). The duration of each test period was 5 minutes, three treatments were applied: (1) (stable): observer horse alone; (2) (arena): observer eating, demonstrator not eating and (3) (arena): observer and demonstrator eating. Measurements taken included latency to approach and ingest feed(s), amount eaten(g), time to consume feed(s) and behavioural observations. Since each food could be novel only once, each of the three flavours was assigned to one of the three treatments within a pair of subjects. Test days were separated by one week; each horse acted as its own control and the horse order was determined by a randomised latin square. The tests were carried out in an indoor arena in two separate pens, each horse had full visual contact with another horse, or in the horse's own stable, companions could be viewed from the door

**Results Social facilitation:** All of the base feed was ingested, no differences existed in the amount consumed of each of the flavoured feeds. An ANOVA and Kruskal-Wallis, where appropriate, revealed no significant difference between the treatment groups and the measures taken (Table 1). It is thought that the methods used were not sensitive enough to establish whether the effects of social facilitation occurred. The time taken to ingest the flavoured feed decreased between the first and the second presentation, regardless of treatment ( $p < 0.05$ ). It is thought that this was due to a neophobic response towards the flavoured feed and not to any one individual flavour.

**Behavioural differences:** No significant differences existed for each of the behaviours scored across the three conditions for both of the trials (Table 1), however there was variation between horses. The six observer horses tested were split into two groups depending upon the amount of vigilant behaviour shown in the presence of another horse or alone. The first 3 horses showed more vigilant behaviour in the presence of another horse than when stabled alone. This pattern was then reversed for the second 3 horses that showed more vigilant behaviour when stabled alone. As vigilant behaviour increased time taken to consume feed decreased and conversely as vigilant behaviour decreased time taken to consume the food increased, this occurred in 3 out of the six horses. These trends were repeated across both of the trials and suggest that sub groups may exist in the population.

**Table 1.** Variables over the three conditions shown as mean  $\pm$  S.E.M

Treatment	Base feed trial			Flavoured feed trial		
	1	2	3	1	2	3
Latency to ingest feed (s)	3 $\pm$ 0	5 $\pm$ 0.5	4.6 $\pm$ 0.3	3 $\pm$ 0	5.5 $\pm$ 0.7	5.1 $\pm$ 0.3
Time to finish feed (s)	160 $\pm$ 12.9	162 $\pm$ 14.8	146 $\pm$ 20.8	258 $\pm$ 18	271 $\pm$ 15.4	245 $\pm$ 29.5
Amount ingested (g)	-	-	-	399 $\pm$ 0.3	396 $\pm$ 2.6	387 $\pm$ 5.7
Vigilant behaviour (head up)	4.4 $\pm$ 0.7	4.7 $\pm$ 0.8	4.1 $\pm$ 0.6	4.3 $\pm$ 0.7	5.0 $\pm$ 0.9	5.9 $\pm$ 1.0

**Conclusion** The results have implications for stable design, as it may be that, for certain horses during competitive activities such as feeding, the close presence of another horse may be either beneficial or stressful. As this is only a preliminary investigation further research is advocated.

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## Training influences problem-solving abilities in dogs (*canis lupus familiaris*)

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**Introduction** One way of testing cognitive abilities (or intelligence") is by using so-called "means-end" tests, where the desired outcome can only be achieved by utilising some means to that end. Pulling a piece of food that is out of reach into reach with the help of an attached string is one of these means-end tasks. The ability of dogs to solve this problem has so far been only tested in 4 studies, all from the 1930s and 40s, with a total of 11 dogs and varying success. Additionally to exploring this basic cognitive ability in pet dogs the study explored whether training, and in particular so-called Clicker training (operant conditioning with secondary reinforcer), had an influence on the performance in this task.

**Method** In this study 16 adult dogs were tested in their homes (one Beagle, one Nova Scotia Duck Tolling Retriever, one Greyhound Mix, three Labrador Retrievers, four Jack Russell Crossbreeds, five Collie Crossbreeds and one Doberman-Bullterrier Cross). Eight dogs were Clicker trained (5 female, 3 male, mean age:  $5.25 \pm 3.49$  years) and the other eight had no experience with the Clicker (2 female, 6 male, mean age:  $5.5 \pm 3.81$  years). Food was attached to a string, the string positioned in a see-through box with a wire-mesh top (see photo), and ca. 10 cm of the string protruded from the box. Every dog had 30 trials, 10 with a short (20 cm) straight string, 10 trials with a long (60 cm) straight string and 10 with the same long string, laid out at a 30 degree angle with the front panel of the box, alternating between left and right set-ups.

**Results** Clicker trained dogs were faster to learn the basic behaviour of pulling the string out of the box (Mann-Whitney  $U = 15$ ,  $p < .05$ , one-tailed). Whereas it took the non-clicker trained dogs on average 39.20 seconds (s.e.s.=15.10) to pull out the food, the initial times for the Clicker trained dogs was 14.03 (s.e.s.=6.93) seconds. When the string was laid out at an acute angle (30 degrees) non-clicker trained dogs tried to dig their way into the box close to the food (see Figure 1), and it took them some time to revert to the learned behaviour of pulling the string (number of proximity errors: mean = 5, s.e.s = 1). Clicker trained dogs (mean = 1, s.e.s = 1) showed significantly less of this so-called proximity error (Mann-Whitney  $U = 9$ ,  $p < .01$ , one-tailed).

**Discussion** Dogs are able to solve this sort of problem but not by understanding the properties of the string but by applying the instinctive behaviour of pawing close to food that is out of their reach. They can learn to retrieve the food by pawing at the string, not close to the food, and this new behaviour is learned faster by dogs that have "learnt to learn". The implications of this research for dog welfare are far reaching: once a dog has learnt how to learn it is easier to train, because new behaviours are acquired faster.



Figure 1: Dog showing the 'proximity error'

## Creep feeding status of piglets can be identified by an automatic marking device

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**Introduction** Creep food intake of suckling piglets varies considerably between individuals (Pajor et al., 1991). The creep feeding status of individual piglets can be monitored by video recording or by combining the weight of the food removed from the electronic dispensers with monitoring by video recording. However, the analysis of videotapes is time-consuming, which limits its widespread use on farm. From a practical standpoint, monitoring the food intake by piglets either before or after weaning is important to provide useful information for a management strategy. Therefore a general, quick and valid method to detect the food intake experience of piglets would be valuable and is needed. The aim of this investigation was to determine if a device that automatically spray-marked piglets at the trough could reliably identify those pigs that had foraged the food in the trough.

**Materials and methods** A creep feeder was constructed with two sets of dye-marking device, which was fitted with a sensor unit and a spraying unit to detect and mark a piglet, respectively. Creep food intake of 103 Large White × Landrace piglets from 12 litters was recorded by litter from day 14 (d14) to d28. Creep feeding behaviour was video recorded on d27-28. If a piglet received a mark on its neck, between the ears or on the shoulder (referred to shoulder), then this was regarded as the piglet having eaten creep food, which was later verified from the video recording. Other positions of the mark and piglets without any mark were regarded as non-feeding incidents. The actual foraging experience of piglets on d27-28 was analysed by video playback. The reliability of the dye-marking device was analysed by Mintab using the chi-square test to derive the association of piglets that actually had eaten creep food on d27-28 and the mark on their shoulder.

**Results** Results showed that average of food intake of suckling piglets from d14 to d28 was low (4.6 g/day\*piglet) and not different between litters ( $P > 0.05$ ). Piglets receiving a mark on their shoulder was significantly ( $\chi^2 = 59.6$ , d.f. = 1,  $P < 0.01$ ) associated with the foraging of creep food (Table 1). The confidence to justify the accuracy of a pig that had the mark and ate the creep food and *vice versa* was over 0.90 (Table .1).

**Table 1** Relationship of foraging creep food and marking on the shoulder of piglets on d27-28 and the probability of accuracy of the dye-marking devices.

	Foraged the food (no.)	Did not forage the food (no.)
Marked on shoulder (no.)	76	8
No mark on shoulder (no.)	1	18

$P < 0.01$  (chi-square test,  $\chi^2 = 59.62$ , d.f. = 1)

Prob (Foraged the food | Marked on shoulder) = 0.905

Prob (Did not forage the food | No mark on shoulder) = 0.947

**Conclusions** It is useful to identify which piglet has or has not foraged the creep food, before weaning is introduced, from the perspective of management. The results suggest that the automatic marking device successfully monitored when suckling piglets had foraged the creep food.

### Reference

Pajor, E. A., Fraser, D. and Kramer, D. L. 1991. Consumption of solid food by suckling pigs: individual variation and relation to weight gain. *Applied Animal Behaviour Science* **32**: 139-155.

# The effect of weaning weight and social challenges on an individual pig's ability to adapt to the postweaning environment

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**Introduction** Feed intake in the days immediately following weaning is both low and variable. This period is critical as low food intakes can lead to reduced digestive efficiency and suboptimal animal welfare. This is of commercial importance as performance around weaning has been shown to impact on the number of days an animal takes to reach a given slaughter weight (Mahan and Lepine 1991). If the variation in an individual's performance could be characterised in terms of feeding behaviour and/or social status then production systems could be designed to optimise growth. In this trial liveweight gain during the late suckling period and liveweight gain, familiarity with penmates and social status (as determined by weight) after weaning were analysed to assess their impact on the performance of the post weaned pig.

**Materials and Methods** Two hundred and fifty five Landrace x (Large White x Duroc) pigs were placed on trial at 10 days of age whilst still suckling their dam. During this time all pigs were offered supplementary creep food. Pigs were weaned at 28 days of age and remained on trial until 56 days of age. At weaning pigs were housed in groups of five in flat deck accommodation. The groups were described as 'light' or 'heavy' according to the pig's weight at weaning; light 5.0-7.0kg, heavy 8.5-11.0kg. Pens of pigs were also described in terms of the individuals familiarity with its penmates; familiar-5 pigs from the same litter of origin, unfamiliar - pigs from 5 different litters of origin. Pigs were weighed at 10 and 28 days of age and then weekly until the end of the trial. Feed refusals were obtained weekly whilst the pigs were in the weaner accommodation. Behavioural observations were made on a weekly basis following weaning. Behavioural observations began immediately following the introduction of fresh feed to the trough.

**Results** The average weaning weight of the pigs described as light was 6.60kg compared to 9.04kg (sem 0.10) for the heavy pigs. When 10 day weights were compared the light pigs were also significantly lighter than the heavy pigs (3.40 vs. 4.46, sem 0.056,  $P < 0.001$ ). At the end of the trial when the pigs were 56 days of age the light pigs were on average 3.8kg lighter than their heavy counterparts (see Table 1). Daily liveweight gain was significantly higher for the heavy pigs than their lighter counterparts throughout the postweaning period. In terms of feeding behaviour, the latency to approach the trough for the first time following weaning was significantly longer ( $P < 0.01$ ) in those pigs described as light when compared to their heavier counterparts although apparent feed intake was not affected at any point during the trial (see Table 1). An individual's familiarity with its penmates had no significant effect on liveweight gain, feed intake or latency to approach the trough. In addition to an individual's weight and familiarity with its penmates, its status within the pen was also analysed. Pigs were described as either lighter than average, similar to the average ( $\pm 0.5$ kg) or heavier than average with respect to the mean pen weaning weight. It was found that this had no significant effect on an individual's growth rate at any time during the trial.

**Table 1** Effect of weight at weaning on postweaning performance

	Light Pigs	Heavy Pigs	s.e.m	P
Wean weight (kg)	6.60	9.04	0.10	
56d weight (kg)	17.35	21.16	0.220	**
DLWG postwean (kg)	0.40	0.45	0.01	*
Latency to approach trough postweaning (s)	2691	371	238	**

**Conclusion** The weight of an individual pig at 10 days of age and at weaning appears to be a good predictor of its performance postweaning. Pigs which are heavier at weaning appear to show a greater motivation to approach the trough immediately following weaning. Therefore the aim for further research in this area must be to try to increase preweaning weight gains of pigs to ease the transition to the weaning environment. In this trial the social status of the individual within a pen or its familiarity with its penmates did not significantly affect postweaning performance.

**Acknowledgements** The authors would like to thank Provimi for their technical help and the provision of the experimental diets.

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## Effects of feeding yeast (*Saccharomyces cerevisiae*) on productive performance and blood components of lactating Holstein dairy cows

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**Introduction** Antibiotics have widely been used in animal feeding. However, because of the growing concern of consumers towards more natural modes of production, interest in the use of direct fed microbials is now considerable. In dairy production, the Yeast culture (*Saccharomyces cerevisiae*) has been studied and used. Therefore, an experiment was conducted to evaluate the effects of different levels of yeast (*Saccharomyces cerevisiae* SC<sub>47</sub>) on productive performance of Holstein dairy cows.

**Materials and methods** In this experiment a balanced change-over design with twelve cows (in early lactation stage), four rations (1-4), three periods (28 day per period), three blocks and four cows per block was employed. Ingredients of the basal diet were alfalfa hay (23.65%), corn silage (17.2%) and concentrate (59.15%) on dry matter basis. The experimental diets 1 to 4 contained 0, 3, 6 and 12 grams of yeast per day, respectively. The rations were fed to cows as total mixed ration (TMR), but yeast was top-dressed on the p.m allotment of ration. The cows were fed individually ad libitum and milked three times per day. Daily milk yield was recorded and samples of the milk were taken twice per week for determination of milk composition, also samples of rumen liquors and blood were taken at the end of each period. All data were analyzed using the SAS statistical package.

**Results** In this experiment dry matter intake and milk yield of cows were not affected by experimental diets. But milk composition including fat, solid non fat and total solid percent were increased by yeast culture ( $p < 0.05$ ). The concentration of milk lactose and protein was not affected by using yeast culture ( $p > 0.05$ ). The averages of 3.2% fat corrected milk with respect to the rations 1-4, 33.25, 30.92, 33.48 and 33.55, for 3.5% FCM 31.61, 29.35, 31.81 and 31.9 and for 4% FCM 29.29, 27.25, 29.47 and 29.53 were calculated. The differences between averages of these FCM were not significant. The pH of cows rumen liquor was not significantly different. Also concentration of glucose, calcium, phosphorus, sodium, cholesterol, triglycerids, urea nitrogen and total protein in plasma of cows received different diets were not different ( $p > 0.05$ ) but concentrations of potassium ( $p = 0.046$ ) and magnesium ( $p = 0.049$ ) in plasma of cows received diet 2-4 were lower than control group and were statistically significant.

**Table 1** Effect of Yeast Culture (*Saccharomyces cerevisiae*) on milk yield, composition, feed intake, rumen pH, blood plasma components.

Item	T-1(control)	T-2(3gr YC/day)	T-3(6gr YC/day)	T-4(3gr YC/day)	SEM <sup>4</sup>
Milk yield, Kg/da	33.38±4.48	31.52±4.83	33.18±4.17	33.02±3.62	0.67
3.5%FCM <sup>1</sup> , Kg/da	31.6±4.87	29.35±3.83	31.81±5.08	31.9±3.88	0.73
Milk Fat, %	3.09±.32 <sup>c</sup>	3.14±.48 <sup>bc</sup>	3.28±.49 <sup>b</sup>	3.39±.34 <sup>a</sup>	0.07
Milk Protein %	2.81±0.17	2.89±0.35	2.94±0.34	2.98±0.32	0.05
Milk SNF <sup>2</sup> , %	8.47±0.35 <sup>b</sup>	8.59±0.54 <sup>a</sup>	8.64±0.43 <sup>a</sup>	8.67±0.46 <sup>a</sup>	0.07
Milk Total solid, %	11.56±0.45 <sup>b</sup>	11.89±0.9 <sup>a</sup>	11.89±0.77 <sup>a</sup>	12.07±0.76 <sup>a</sup>	0.12
DMI <sup>3</sup> , Kg/day	19.07±2.51	18.73±2.48	18.99±1.94	19.68±2.2	0.37
Rumen pH	6.86±0.19	6.8±0.11	6.87±0.16	6.88±0.1	0.02
Blood plasma composition					
Total protein, g/dL	7.77±2.24	8.33±.73	7.41±2.34	7.76±1.44	1.44
Glucose, mg/Dl	58.33±21.1	62.56±5.39	56.33±12.6	66.89±10.12	1.34
K, mmol/L	8.15±7.7 <sup>a</sup>	3.30±.77 <sup>b</sup>	2.56±0.25 <sup>b</sup>	2.69±0.26 <sup>b</sup>	1.26
Mg, mmol/L	3.7±4.1 <sup>a</sup>	1.23±0.16 <sup>b</sup>	1.24±0.3 <sup>b</sup>	1.23±0.08 <sup>b</sup>	0.41
Na, mmol/L	161/7±43.5	145.22±30.4	154±32.4	161.7±30.5	4.35

means in the same row with different superscripts within a trial differ ( $p < 0.05$ ).

### Conclusion

The results of the present study demonstrate that the addition of Yeast Culture in the diet of early lactation Holstein dairy cows was beneficial in improving milk fat, SNF and total solid. YC reduced K and Mg concentrations of blood plasma. milk yield, 3.5%FCM, DMI, rumen pH, another blood plasma composition were unaffected. in present study optimum level of YC was 6 gr /day/head cow.

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## The effect of ammoniated and roasted barley on rumen pH, milk yield and milk composition of lactating dairy cows

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**Introduction** Cereal grains can provide the major source of energy in diets in order to meet the nutrient requirements of high producing dairy cows. However the amount of starch that can be included in the diets of dairy cows is limited particularly if starch is rapidly fermented such as barley starch. Reduction of feed intake, rumen pH, milk fat test, microbial growth and other metabolic disorders are expected if ruminally degradable starch is fed in amount that cant be efficiently metabolized by rumen microbes. Various techniques for processing barley grain have been developed to decrease the degradability of dry matter in rumen without reducing its extent of digestion. McNiven (1995) showed roasting of barley is more effective treatment. The objective of this experiment was to study of effects the roasting and ammoniation of barley grain on rumen pH, feces pH, milk yield and milk composition in dairy cows.

**Materials and Methods** Twelve multiparous Holstein cows in early lactation [52 (s.d. 10) DIM] with similar body conditions were assigned to four rations in a balanced change over design (with three 28-d period and 7-d interval between periods). Cows averaged 589 (s.d. 37.5) kg BW, and yielded 29.5 (s.d. 2.45) kg milk/d. To prepare processed barley, barley grain was mixed with 40g/kg urea solution with 100:75 ratio (barley:water) and ensiled for 60 days. Barley was also roasted at 115-120°C. Rations (TMR), based on concentrate and alfalfa hay (0.63:0.37 dry matter basis) were offered *ad libitum* twice daily and had the following composition: DM 910g/kg, NE<sub>1</sub> 1.59 Mcal/kg DM, CP 159 g/kg DM, RUP 53g/kg DM, NDF 340g/kgDM, ENDF 213g/kgDM. Four ration were similar and only difference between rations was type of barley so that in diet 1 barley was unprocessed (UNB), in diet 2 barley was ammoniated (AMB), in diet 3 barley was roasted (ROB) and in diet 4 barley was roasted and then ammoniated (RAB) Milk yield was measured daily and its composition was analysed twice in week. Samples of rumen fluid were drawn on d 21 of each period via the stomach tube at 3 h after feeding and rumen pH was determined. Data was analysed as a four-treatment change over design experiment using analysis of variance.

**Results** Milk yield (crude and 4%FCM) and milk fat concentration was increased ( $P<0.01$ ) with roasted and ammoniated barley (Table 1). An increased supply of starch to the small intestine and hence more glucose uptake by mammary glands increased milk yield. Higher ratio of acetate:propionate and increasing of rumen pH, increased milk fat concentration too. Milk protein concentration was not affected by processed barley. However cows that consumed diet 2 had higher milk protein concentration, probably because of higher microbial protein synthesis. DMI per kg FCM was reduced by processed barley. Rumen pH was increases by processed barley particularly with roasted-ammoniated barley.

**Table 1** Treatment effects on feed intake, rumen pH and milk production

	Diet				SEM	SIG.
	UNB	AMB	ROB	RAB		
DM intake (kg/d)	23.07 <sup>c</sup>	23.80 <sup>b</sup>	24.95 <sup>a</sup>	24.26 <sup>b</sup>	0.1864	**
DM intake (kg/kg FCM)	1.10 <sup>a</sup>	1.03 <sup>b</sup>	1.03 <sup>b</sup>	1.0 <sup>b</sup>	0.0134	**
Milk yield (kg/d)	25.11 <sup>c</sup>	26.33 <sup>b</sup>	27.22 <sup>a</sup>	26.79 <sup>ab</sup>	0.1978	**
4%FCM (kg/d)	20.94 <sup>b</sup>	23.10 <sup>a</sup>	24.12 <sup>a</sup>	24.15 <sup>a</sup>	0.3681	**
Milk composition (g/kg)						
Fat	29.0 <sup>b</sup>	32.0 <sup>a</sup>	32.80 <sup>a</sup>	33.50 <sup>a</sup>	0.0677	**
Protein	31.10	33.0	29.60	30.60	0.0513	ns
Milk component yield (g/d)						
Fat	726 <sup>c</sup>	837 <sup>b</sup>	882 <sup>a</sup>	896 <sup>a</sup>	0.0109	**
Protein	779 <sup>c</sup>	866 <sup>a</sup>	804 <sup>bc</sup>	818 <sup>b</sup>	0.0121	*
Rumen pH	6.02 <sup>c</sup>	6.22 <sup>b</sup>	6.32 <sup>ab</sup>	6.43 <sup>a</sup>	0.0383	**
Faeces pH	6.12	6.13	6.12	6.20	0.0418	ns

\* $P<0.05$  , \*\* $P<0.01$

**Conclusions** The results of the present study demonstrate that ammoniation and/or roasting of barley grain can effectively increase percentage of milk fat and milk yield and reduce the incidence of rumen acidosis in milking cows.

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# Prediction of nitrogen degradability in grass silages using nutrient concentration and fermentation variables

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**Introduction** Nitrogen (N) degradability is a key variable in protein rationing systems but there is little information in the literature to predict it in a feed. The objective of the present study was to develop equations to calculate N degradability in grass silages using nutrient concentration and fermentation data.

**Material and methods** A total of 136 grass silages, obtained from commercial farms across Northern Ireland, were evaluated for N degradability. Each silage was incubated in triplicate in the rumens of three steers, fitted with permanent ruminal cannulae, for respectively 0, 6, 12, 24, 48 and 72 hours. The animals were offered an average grass silage *ad libitum* for 21 days before the commencement of N degradability measurements. These measurements were in a randomized-block design with the three steers as the three blocks. There were a total of 17 periods (8 silages/period) each of two weeks duration. In each period, a total of 24 bags (3\*8 silages) were incubated in the three steers for each time interval (i.e., 6, 12, 24, 48 or 72 hours). 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. Silage DM concentration was determined on an alcohol-toluene basis, which was subsequently used as a basis of expressing all nutrient concentrations in silages. N degradability at r (rumen outflow rate) = 0.02/hour ( $P_{0.02}$ ) was calculated from the readily soluble proportion of N (a), potentially degradable N (b) and the fractional degradation rate of b (c). Details on the procedure and calculation were reported by Dawson and Steen (2000). Linear and multiple regression equations were used to relate  $P_{0.02}$  to nutrient concentrations and fermentation variables in silages.

**Results** There was a wide range in silage quality. The ranges and mean in DM were 155-413 and 219 g/kg, CP 79-212 and 133 g/kg DM, NDF 413-662 and 544 g/kg DM, pH 3.5-5.5 and 4.2, and ammonia-N/total-N 45-385 and 123 g/kg, respectively. The mean (s.d.), minimum and maximum data for a values were 0.674 (0.0577), 0.537 and 0.818; b values 0.235 (0.0511), 0.116 and 0.343; c values 0.079 (0.0196), 0.040 and 0.174; and  $P_{0.02}$  0.859 (0.0351), 0.750 and 0.934, respectively.  $P_{0.02}$  was positively related to concentrations of CP, ether extract, ash, lactic acid and ethanol, and soluble-N/total-N (SN/N) and non-protein-N (NPN)/total-N ( $P < 0.01$  or less), while negatively related to NDF, ADF, lignin and WSC concentrations ( $P < 0.05$  or less). Prediction equations for  $P_{0.02}$  are presented in Table 1. All relationships were significant ( $P < 0.001$ ) and each predictor had a significant effect on the relationship ( $P < 0.05$  or less).  $P_{0.02}$  fitted better to each of CP, soluble CP (SCP) and non-protein CP (NPCP, =  $NPN * 6.25$ ) in a quadratic line (eqs. (1b), (2b) and (3b)) than in a linear line (eqs. (1a), (2a) and (3a)), with  $R^2$  values being increased by 0.04-0.05. The positive relationship between  $P_{0.02}$  and CP may reflect that CP was positively related to SN/N ( $P < 0.001$ ) but negatively to NDF ( $P < 0.001$ ).  $P_{0.02}$  could also be predicted using NDF, ADF and lignin (eqs. (4a)-(6a)), but  $R^2$  values were lower than those using protein fractions. The combination of CP, SN/N, DM and NDF to predict  $P_{0.02}$  improved the prediction accuracy with the  $R^2$  being increased to be 0.81 (eq. (7)). The addition of fermentation variables as predictors to eq. (7) marginally increased  $R^2$  values (eqs. (8) and (9)), although they each had a significant effect on the relationship. A quadratic equation was developed to calculate N degradability ( $P_x$ ) at any level of rumen outflow rate (0.03 – 0.12/h) (x):

$$P_x = P_{0.02} * (6.870x^2 - 2.024x + 1.035) \quad (R^2 = 0.998)$$

**Table 1** Prediction equations for  $P_{0.02}$  (data in brackets are s.e. values; unit for all predictors is kg/kg or kg/kg DM; SCP – soluble CP, NPCP – non protein CP, SN/N – soluble-N/total-N)

	Linear equations	$R^2$	Eqs.	Quadratic equations	$R^2$	Eqs.	
CP:	$P_{0.02} = 1.107_{(0.079)} x + 0.713_{(0.011)}$	0.59	(1a)	$= -7.200_{(2.110)} x^2 + 3.128_{(0.597)} x + 0.575_{(0.042)}$	0.63	(1b)	
SCP:	$P_{0.02} = 1.716_{(0.100)} x + 0.729_{(0.008)}$	0.69	(2a)	$= -20.469_{(3.990)} x^2 + 4.993_{(0.645)} x + 0.605_{(0.025)}$	0.74	(2b)	
NPCP:	$P_{0.02} = 1.600_{(0.106)} x + 0.748_{(0.008)}$	0.63	(3a)	$= -20.570_{(4.360)} x^2 + 4.628_{(0.648)} x + 0.643_{(0.023)}$	0.68	(3b)	
NDF:	$P_{0.02} = -0.476_{(0.043)} x + 1.119_{(0.024)}$	0.47	(4a)				
ADF:	$P_{0.02} = -0.484_{(0.074)} x + 1.030_{(0.026)}$	0.24	(5a)				
Lignin:	$P_{0.02} = -1.527_{(0.168)} x + 0.933_{(0.009)}$	0.38	(6a)				
<b>Multiple regression equations</b>						$R^2$	Eqs.
$P_{0.02}$	$= 0.610_{(0.072)} CP + 0.258_{(0.029)} SN/N - 0.140_{(0.032)} DM - 0.265_{(0.034)} NDF + 0.807_{(0.031)}$					0.81	(7)
$P_{0.02}$	$= 0.705_{(0.078)} CP + 0.251_{(0.028)} SN/N + 0.132_{(0.048)} \text{Lactic} - 0.134_{(0.032)} DM - 0.196_{(0.042)} NDF + 0.751_{(0.036)}$					0.82	(8)
$P_{0.02}$	$= 0.751_{(0.081)} CP + 0.247_{(0.028)} SN/N + 0.209_{(0.056)} \text{Lactic} + 1.755_{(0.660)} \text{Propionic} + 1.950_{(0.779)} \text{Propional}$ $- 0.108_{(0.038)} DM - 0.214_{(0.041)} NDF - 0.701_{(0.250)} \text{Acetic} + 0.750_{(0.036)}$					0.83	(9)

**Conclusion** A number of equations have been developed to predict N degradability in grass silages using nutrient concentration and fermentation variables.

**Reference** Dawson, L. E. R. and Steen, R. W. J. 2000. Relationship between dry matter, fibre and nitrogen degradation characteristics of silage and silage intake of steers. *Animal Science* **70**: 537-546.

# Effect of dietary protein and energy concentration on the fattening performance of buffalo male calves in southern Iran

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**Introduction** During the last decade, development of the buffalo industry has been undertaken in Iran. The prospects for beef production from buffalo management have shown to be successful under local environments. Buffaloes reared under feedlot conditions with suitable diets have the potential to produce high quality carcass (Udeybir and Mandal, 2001). In terms of nutrition, studies are needed for the validation of nutrient requirements (especially protein and energy) for different physiological stages. A system to monitor the nutritional status of buffaloes would be beneficial to reduce losses and maximize efficiency of nutrient utilization. To optimise protein efficiency and reduce nitrogen wastage, diets need to be formulated to provide optimum nitrogen concentration for maximum rumen microbial yield and growth. However, limited work has been reported on nutrient requirements of growing and fattening buffalo calves. The objective of this work was to study the response of Iranian buffalo male calves to the different levels of energy and protein in the diet from 12 to 18 months of age.

**Material and Methods** A 3×3 factorial completely randomized design was conducted in which 27 yearling buffalo male calves with initial live weight of 201 ± 4 were selected from the buffalo farms in Khoozestan province in southern Iran. The animals were individually housed and randomly allocated into 9 treatment groups of three animals each. Three levels of energy (E<sub>1</sub>, E<sub>2</sub>, E<sub>3</sub>) with three levels of crude protein (P<sub>1</sub>, P<sub>2</sub>, P<sub>3</sub>) were formulated to provide 90,100 110% requirements for 750 g. body weight gain of steers derived from NRC (1976) beef cattle requirements (Table 1). Diets consisted of alfalfa hay, wheat straw, barley grain, wheat bran, sugar beet pulp, molasses, urea and mineral supplements and offered *ad libitum* as total mixed ration. The experiment carried out for 6 month where live weight changes were obtained by direct weighing of the animals every month and ended by slaughtering the calves for carcass measurements.

**Results** As indicated in Table 2, the dry matter intake (DMI) of the low energy diets was slightly higher than those with high energy contents, but no differences of DMI was observed with different levels of protein. However the differences in DMI among the treatments was not significant (P>0.05). Average daily gain was significantly (P<0.05) varied among the diets. The significantly (P<0.05) higher daily gain was obtained when animals received medium energy diets that was in accordance with the NRC beef cattle requirements. In addition the feed conversion ratio (kg of DMI per kg of body weight gain) was significantly (P<0.05) lower when the animals received medium energy diets. The dressing yield as well as the meat percentage was not affected by the type of the diet, but abdominal fat as a percentage of total carcass was significantly (P<0.05) higher in medium and high energy diets.

**Table 1** Concentration of the energy and protein in the experimental diets.

	Diets								
	E <sub>1</sub> P <sub>1</sub>	E <sub>1</sub> P <sub>2</sub>	E <sub>1</sub> P <sub>3</sub>	E <sub>2</sub> P <sub>1</sub>	E <sub>2</sub> P <sub>2</sub>	E <sub>2</sub> P <sub>3</sub>	E <sub>3</sub> P <sub>1</sub>	E <sub>3</sub> P <sub>2</sub>	E <sub>3</sub> P <sub>13</sub>
ME (Mcal/kg DM)	2.14	2.14	2.14	2.38	2.38	2.38	2.62	2.62	2.62
Crude Protein (g/100gDM)	11.81	13.12	14.43	11.81	13.12	14.43	11.81	13.12	14.43

**Table 2** Responses of the animals to the various levels of energy and protein diets.

Item	Treatments									s.e.	sign.
	E <sub>1</sub> P <sub>1</sub>	E <sub>1</sub> P <sub>2</sub>	E <sub>1</sub> P <sub>3</sub>	E <sub>2</sub> P <sub>1</sub>	E <sub>2</sub> P <sub>2</sub>	E <sub>2</sub> P <sub>3</sub>	E <sub>3</sub> P <sub>1</sub>	E <sub>3</sub> P <sub>2</sub>	E <sub>3</sub> P <sub>13</sub>		
DM Intake (kg/d)	5.65	6.43	5.37	6.39	5.96	6.09	5.08	4.81	5.01	0.79	ns
Weight Gain (g/d)	694 <sup>d</sup>	875 <sup>abc</sup>	662 <sup>d</sup>	1034 <sup>a</sup>	935 <sup>ab</sup>	977 <sup>a</sup>	755 <sup>bcd</sup>	703 <sup>cd</sup>	732 <sup>cd</sup>	98	*
F.C.R. <sup>1</sup>	8.15 <sup>a</sup>	7.4 <sup>ab</sup>	8.13 <sup>a</sup>	6.16 <sup>b</sup>	6.35 <sup>b</sup>	6.24 <sup>b</sup>	6.72 <sup>ab</sup>	6.79 <sup>ab</sup>	6.84 <sup>ab</sup>	0.58	*
Carcass Weight (kg)	157	181	156	188	184	187	171	160	159	23	ns
Carcass Percentage	47.9	49.9	48.8	48.0	49.4	49.8	49.3	48.1	48.1	3.06	ns
Abdominal fat (%) <sup>2</sup>	12.1 <sup>b</sup>	14.7 <sup>b</sup>	12.7 <sup>b</sup>	17.4 <sup>a</sup>	15.5 <sup>ab</sup>	13.3 <sup>ab</sup>	14.1 <sup>ab</sup>	12.3 <sup>b</sup>	14.2 <sup>ab</sup>	4.7	*
Meat (%) <sup>2</sup>	67.33	66.89	67.74	64.44	65.65	67.33	68.14	68.05	66.42	5.4	ns

Means within the same row with different superscript are significantly different (P<0.05), \* (P<0.05).

<sup>1</sup>Feed conversion Ratio (kg DMI/kg Live weight gain), <sup>2</sup>Percent of total carcass

**Conclusion** It may be concluded that the optimum growth rate and feed efficiency of yearling buffalo male calves can be obtained by providing 2.38 Mcal/kg of dietary metabolisable energy and around 12% of crude protein.

**Acknowledgements** This research was supported by the Animal Science Research Institute of I.R.Iran and Research Center for Natural Resources and Animal Production of Khoozestan Provinces, I.R.Iran.

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## Effect of salts of sulphite on yeast numbers and temperature of whole-crop wheat silage

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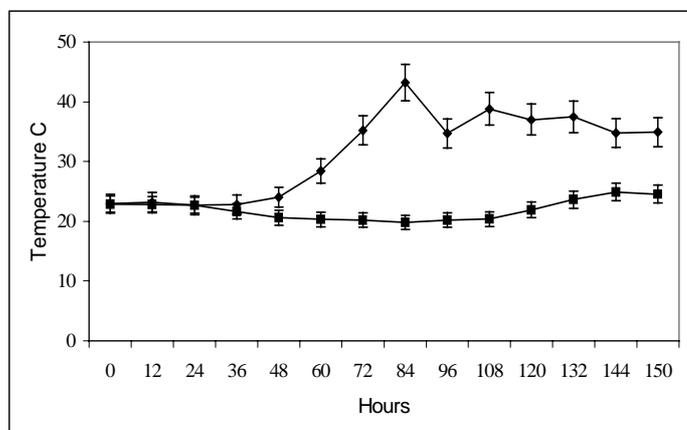
**Introduction** Maize and whole crop silages are particularly susceptible to spoilage from aerobic micro-organisms. As a consequence, the maintenance of aerobic stability of silage during the feed-out phase is important in the preservation of silage quality and animal health (Scudamore and Livesey, 1998). The aim of this study was to monitor the effect of low levels of salts of sulphite on the stability of whole crop wheat silage, pH, temperature and laboratory culture of yeast.

**Materials and methods** Using sterile surgical gloves 1.5 kg of WSW silage treated with sulphite salts (SS) at 0.5kg per tonne (equivalent 125ppm/t SO<sub>2</sub>) and 1.5 kg WCW silage with no additional SS (NoSS) were prepared. Each treated silage was loosely packed into open plastic containers (4 lts volume) in triplicate. All containers were punctured with 20, 0.5-cm diameter holes to allow air movement without extensive sample drying prior to sterilisation. The containers were then stored using a mini-silo technique. A 5cm layer of polystyrene insulation packaging was placed around and on top of the silage allowing the ingress of air. Containers were stored at ambient temperature (21 °C) in a forced draft incubator. Forage crop samples (10 g) were massaged by hand with 90 ml of Maximum Recovery Diluent for 1 minute, and 10<sup>-1</sup> to 10<sup>-7</sup> serial dilutions were made in 0.85% NaCl solution. In triplicates, 0.02 ml suspension from each dilution was spread on agar plates and incubated in an anaerobic box at 22 °C for 2 to 3 days. Yeast was cultured on rose bengal and chloramphenicol agar, distinguished from mould or bacteria by colony and morphological appearance and counted. Treatments were compared using ANOVA.

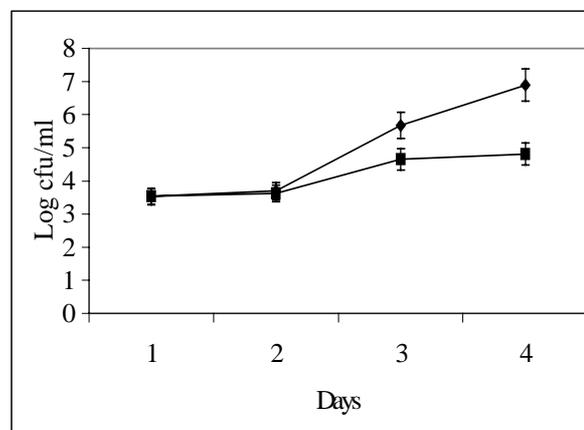
### Results

**Table 1** Changes in temperature of whole crop silage with additional sulphite salts (SS) or with no additional sulphite salts (NoSS) on exposure to air over 150 h

	SS	NoSS	s.e.m.	Sig
Time to 2 °C rise above ambient temperature (h)	119.95	55.90	2.277	***
Time to peak temperature (h)	128.64	82.89	1.547	***
Maximum temperature rise (°C)	6.76	10.90	0.372	***
Total heat generated (°C)	39744.00	54938.00	1171.000	***



**Figure 1** Temperature change (°C) of whole-crop wheat silage with additional sulphite salts (- □-) or with no additional sulphite salts (- ◆-) once exposed to air



**Figure 2** Yeast count of whole-crop wheat silage with additional sulphite salts (- □-) or with no additional sulphite salts (- ◆-) once exposed to air

**Discussion** The addition of sulphite salts, even at low levels (0.5kg per tonne WCW silage or >125ppm/t SO<sub>2</sub>, significantly reduced the time required to increase silage temperature by 2 °C above ambient temperature, the time to maximum increase in temperature, the total heat generated and the numbers of yeast.

**Conclusion** The addition of sulphite salts reduced heating, yeast proliferation and aerobic spoilage of WCW silage.

# The effect of salts of sulphite on intake levels of urea treated whole-crop wheat silage fed to grazing Holstein-Friesian dairy cattle

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**Introduction.** Forage supplementation is a strategy sometimes used in dairy production systems to achieve increased dry-matter (DM) intakes in grazing dairy cows. During periods of low grass growth rates, often because of low rainfall, such intakes are not possible from grass alone (Phillips, 1988) and buffer feeds are used. More recently, whole crop cereal and maize silage have been utilised. These forage crops are susceptibility to aerobic deterioration which can result in significant losses in quality (Sanderson, 1993; Weinberg *et al.*, 1993) however the addition of salts of sulphite, typically using in the food industry, can prevent aerobic deterioration. The aim of this study was to measure the effect of low levels of salts of sulphite on the stability of whole crop wheat silage used as a buffer feed on feed intake and milk yield.

**Materials and methods.** Between 10<sup>th</sup> June and the 22<sup>nd</sup> of August 2002, 40 Holstein-Friesian cows were allocated, at 60 days postpartum, in pairs according to date of parturition, milk yield, live weight (LW), condition score and parity to one of two dietary treatments. Twenty cows were offered whole crop wheat (WCW) silage treated with the sulphite salts at 0.5kg per tonne (sufficient to supply not less than 125ppm/t SO<sub>2</sub>) and twenty cows were offered WCW silage with no additional sulphite salts. The experimental diets were offered for a period of 42 days, which included a 14-day diet acclimatisation period followed by a 28-day measurement period. The treatment diets were offered twice daily for 55-60 minutes following milking and individual feed intake was recorded using the Griffith Elder feeding facilities. All cows were observed while grazing the same pasture. Pre-treatment milk yield and composition were measured for two weeks prior to feeding of treatment diets and used as covariates in the data analysis, using ANCOVA with animal and diet as factors in the model.

## Results

**Table 2.** Mean feed intake levels of cows supplemented with whole crop silage with additional sulphite salts (SS) or with no additional sulphite salts (No SS)

Mean	SS	No SS	s.e.m.	Sig
Whole-crop silage intake (kg DM/d) †	6.9	5.9	0.19	*
Compound feed intake (kg DM/d)	5.1	5.1	0.00	NS
Mean grazing time (min/d)	263.5	296.0	6.911	***
Biting rate (bites min <sup>-1</sup> )	61.4	61.4	0.22	NS
Ruminating time (min d <sup>-1</sup> )	546.6	530.8	3.353	***
Estimated ‡				
Total feed intake (kg DM/d)	17.9	17.9	0.15	NS
Total ME intake (MJ/d)	221.3	217.4	1.056	**
Herbage intake (kg DM/d)	5.9	6.9	0.03	***
Estimated bite size (g DM)	0.37	0.40	0.008	**
Herbage intake rate (g DM min <sup>-1</sup> )	22.6	24.5	0.48	**
Milk yield (kg/d)	33.3	32.9	0.52	NS
FCM yield (kg/d) †	32.1	31.7	0.50	NS
Milk fat (g/kg)	40.5	42.1	0.07	NS
Milk protein (g/kg)	33.6	33.6	0.03	NS
Milk lactose (g/kg)	47.2	47.1	0.01	NS
Milk urea (g/kg)	86.5	90.4	0.17	NS

†DM content of forage is by toluene distillation; DM content of concentrates and herbage is on oven-dry basis. ‡ME = metabolizable energy estimated from modified acid detergent fibre.

**Discussion.** The addition of salts of sulphite to WCW significantly increased whole-crop silage intake and reduce mean grazing and ruminating time (min/d). However, there was no significant effect on milk yield.

**Conclusion.** The addition of salts of sulphite to WCW significantly increased DM intake, but not milk yield.

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## Effect of weaning at 6 or 8 weeks old on the performance of dairy-bred beef calves

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**Introduction** Artificial rearing is a common practice for rearing calves from the dairy herd destined for beef production. In commercial practice calves are typically weaned from 5 to 9 weeks old. There are four criteria that can be used to determine weaning time: - age, compound feed intake, liveweight, and, milk price and quota policy. Late weaning systems are based on the theory of giving the calf the best possible start in life, but are costly with high milk intakes (Davis and Drackley, 1998). Hence emphasis is usually placed on early weaning of the calf and encouraging concentrate intake. The objective of this study was to determine the effect of weaning at either 6 or 8 weeks old on the performance dairy-bred beef calves.

**Materials and Methods** Thirty-six Belgian Blue and Limousin cross Holstein bull and heifer calves were assigned in a randomised block designed experiment to weaning at either 6 or 8 weeks of age. The calves started the trial at 1 day of age and were individually penned on straw. From days 1 to 14 and day 15 to 7 days prior to weaning they were fed 4 and 6 litres per day of milk respectively. At 7 days prior to weaning they were fed 3 litres of milk. The milk (DM 140g/kg, 37.5g butterfat/kg, 31.1g protein/kg, 44.3g lactose/kg) was split into two daily feeds. From day 6 the calves received *ad libitum* concentrates (DM 864 g/kg; 13.4 MJ ME/kg DM; 229g crude protein/kg DM) plus water. The calves were moved into group pens at 8 weeks old. The data was analysed by ANOVA with calves blocked according to sex and breed.

**Results.** There were no significant differences in calf weaning weight or daily liveweight gain with weaning at either 6 or 8 weeks old. There was a trend for higher DLWGs from 6 to 8 weeks and 8 to 12 weeks with calves weaned at 6 weeks of age. Concentrate feed intakes were significantly higher from birth to weaning with 8 week weaning but there was an increase in total concentrate feed intake from birth to twelve weeks old with calves weaned at 6 weeks old. There were no differences in the health or condition of the calves.

**Table 1** Effect of weaning age on liveweight (kg)

Weaning age (weeks)	6	8	s.e.d	Significance
Birth weight	44.83	43.67	2.091	NS
2 week weight	50.00	48.02	2.051	NS
4 week weight	59.51	58.15	2.247	NS
6 week weight	69.36	68.71	2.552	NS
8 week weight	79.88	77.63	3.026	NS
12 week weight	110.92	105.12	4.134	NS

NS = not significant

**Table 2** Effect of weaning age on DLWG (kg)

Weaning age (weeks)	6	8	s.e.d	Significance
Birth - 2 week DLWG	0.372	0.310	0.0465	NS
2 - 4 week DLWG	0.686	0.738	0.0648	NS
4 - 6 week DLWG	0.703	0.743	0.0519	NS
6 - 8 week DLWG	0.751	0.607	0.0750	0.059
8 - 12 week DLWG	1.107	0.979	0.0618	0.052
Birth - 12 week DLWG	0.780	0.668	0.0744	NS

**Table 3** Concentrate feed intakes (kg/head)

Weaning age (weeks)	6	8	s.e.d	Significance
Intake Birth – weaning	19.69	37.25	5.073	***
Intake Weaning – 12 weeks	104.86	76.47		
Total intake Birth – 12 weeks	124.55	113.72		

\*\*\*P<0.001

Milk intakes were 203 and 287 litres per calf for the 6 and 8 week weaning ages respectively. Based on the prices prevailing at the time of the study with whole milk costing 18p/litre and concentrates costing £150/t, the total feed costs per calf were £55.21 and £68.71, and the feed costs per kg LWG were 83 and 113p for the 6 and 8 week weaning ages respectively.

**Conclusions** Weaning calves early at 6 weeks old has no significant effect on performance to 12 weeks of age compared to weaning at 8 weeks old. Calves weaned at 6 weeks old consumed more concentrates. Early weaning at 6 weeks old significantly reduced calf rearing costs.

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# Validation of a model to predict live weight and estimate dry matter intakes of individual dairy cows on commercial farms

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**Introduction** A method to estimate total dry matter intake (DMI) of individual dairy cows on commercial farms was developed to aid nutritional research on commercial farms (Wicks, 2001). Recently a second validation of the model was carried out using cows from the Agricultural Research Institute of Northern Ireland (ARINI) dairy herd. The results from the validation process at Wye and ARINI are shown and the mean square prediction error (MSPE) of the model for live weight prediction and DMI estimation were calculated.

**Materials and Methods** Live weight was measured and predicted for: the Wye College Dairy Herd (n=114) on 3 occasions, weekly during early lactation for 40 cows on trial at Wye, and for the ARINI herd (n=253) (including both Holstein and Norwegian genotypes). Live weights were predicted using the model described by Wicks, (2001), which included 4 parameters easily obtainable on-farm: body condition score, height at withers, parity and stage of gestation. Dry matter intake data were measured and predicted from 40 group fed cows at Wye in 1997, and 40 individually fed cows at ARINI in 2002, DMI was estimated from total Metabolisable Energy (ME) requirements and ME density of the diet. The mean square prediction error (MSPE) (Bibby and Toutenburgh, 1977) was used to assess the accuracy of the model.

**Table 1** Actual (A) and Predicted (P) Live Weights and MSPE

		A	s.d. <sub>(A)</sub>	P	s.d. <sub>(P)</sub>	R <sup>2</sup> (%)	MSPE (kg <sup>2</sup> )
Wye	Autumn – 1997	619.8	87.45	604.1	77.35	78	1905
	Spring – 1998	635.3	78.68	630.6	59.43	71	1865
	Winter – 1999	623.9	80.19	612.1	61.59	63	2527
Wye*	Group Fed	567.8	76.07	568.0	59.57	71	1694
ARINI	February - 2002	556.7	67.50	582.3	64.19	58	2575

**Results** The variation in prediction errors for data collected from Wye was 41.2 to 50.3 kg, calculated from the MSPE shown in Table 1. The MSPE for data collected from the ARINI dairy herd was 2575 kg<sup>2</sup> (prediction error was 50.7

s.d. is the standard deviation, and MSPE is the mean square prediction error.  $MSPE = (A-P)^2 + S^2_P (1-b)^2 + (1-r^2)S^2_A$ , where A is the actual live weight, and P is the predicted. Wye\* Average of results over first 15 weeks of lactation.

kg) (Table 1). Analysis of variance showed that there was no significant difference between the actual and predicted live weights ( $P=0.949$ ). The live weight prediction error equated to a ME requirement for maintenance of 4.60 MJ/d or an intake of 0.41 kg DM/d (ME density of the diet = 11.5 MJ/kg DM). The DMI prediction error was reduced to 1.5 kg/d, from 2.2 kg/d, (ARINI data) when an updated value of ME for maintenance was used (Yan, *et al.*, 1997). The DMI prediction error, for data from Wye, was 2.1 kg/d.

**Table 2** Actual (A) and Predicted (P) DMI, and MSPE

	A	s.d. <sub>(A)</sub>	P	s.d. <sub>(P)</sub>	R <sup>2</sup> (%)	MSPE (kg <sup>2</sup> )
Wye*	19.8	2.04	19.9	1.59	26	4.00
ARINI 1	18.7	1.51	16.9	1.22	19	4.79
ARINI 2	18.7	1.51	18.5	1.22	19	2.21

s.d. is the standard deviation, and MSPE is the mean square prediction error.  $MSPE = (A-P)^2 + S^2_P (1-b)^2 + (1-r^2)S^2_A$ , where A is the actual intake, and P is the predicted. Wye\* and ARINI 1 DMI calculated using AFRC 1993 values for ME for maintenance, ARINI 2 calculated using an updated value for ME for maintenance (Yan, *et al.*, 1997)

**Conclusions** The results have shown that it is possible to use the method described by Wicks (2001) to predict live weight and estimate DMI satisfactorily on commercial farms from ME requirements, body condition score, height at withers, parity and stage of lactation. The updated value of ME for maintenance (Yan, *et al.*, 1997) improved the accuracy of prediction.

**Acknowledgements** The authors would like to thank Phil Drury for help with collection of data from the Wye College Dairy Herd, and the technical team at ARINI for provision of data from the ARINI Dairy Herd. The authors would also like to acknowledge the support of the South of England Agricultural Society.

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## A comparison of silage and dried common reed (*phragmites australis*) for finishing male calves

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**Introduction** The common reed (CR) is one of the plants which grows in some area of Iran and other world countries especially in coastal wetlands. Feed resource restriction is the most problem in animal production in some area and native plants have a key role. Ensiling may improve the quality of crop or decrease by high fermentation. These effects depend on stage of growth, chemical composition, dry matter and nutrients of plant, and final effect is not as same as for all crops. For understanding the effect of ensiling on quality of each plant must be investigate. For this reasons goals of this experiment was investigation on various methods of utilization of CR for finishing male calves and comparison with alfalfa.

**Materials and methods** 28 native (Sistani) male calves (12-14 month old, ave. 172.97kg) were used according to Completely Randomized Design with 7 replications in each of 4 treatments. A total mixed ration based on concentrate and forage or silage (0.58:0.42 DM basis) was offered through the study (170 days) ad libitum. Energy, protein and physical form of rations were similar (CP 123g/kg, ME 10.26MJ/kg) and non concentrate part of ration were different as follows: sun cured alfalfa (SA) or sun cured common reed (SCR) or common reed silage which 35g/kg (DM basis) molasses were added as silage additives (MCRS) or common reed silage which 50g/kg (DM basis) molasses and 15g/kg (DM basis) urea were added as silage additives (MUCRS). Average daily gain (ADG), dry matter intake and body measurements were recorded through the experiment and carcass traits were recorded at the end of experiment.

**Results** Some results of this experiment are shown in Table 1. Calves that consumed MCRS had a minimum average daily gain (ADG) and the differences between treatments were significant ( $p < 0.01$ ) but the difference between carcass meat was not significant ( $p > 0.05$ ) and SCR treatment had lowest carcass fat. By calculating Euclidean Distance for important finishing traits (dressing percentage, daily gain and feed conversion) which shows in following, and using the data of Steen and Challett (1988), concludes that CR treatment is the nearest to SA treatment (as a good roughage source).

**Table 1** Mean finishing trait.

Treatment	SA	SCR	MCRS	MUCRS	sem
ADG(kg/day)	1.01	0.87	0.83	0.84	0.09
Meat (kg/kg carcass weight)	0.72	0.73	0.68	0.72	0.03

**Table 2** Euclidean Distance

	SA	SCR	MCRS	MUCRS
SA	-	2.11	4.11	2.95
SCR	2.11	-	3.55	2.42
MCRS	4.11	3.58	-	3.20
MUCRS	2.95	2.42	3.20	-

Effect of treatment on ADG is significant ( $p < 0.01$ )

**Conclusion** The results of present study demonstrate (and with respect to economic consideration) the use of sun cured common reed is better than common reed silage and a good roughage source for finishing male calves.

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# Inclusion of varying levels of papaya (*Carica papaya*) pomace in concentrate mixtures on nutrient utilization in native male buffaloes

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**Introduction** India occupies 2<sup>nd</sup> position in the world with a total fruit production of 42 million tonnes. Papaya (*Carica papaya*) fruit production in India is 1.3 million tonnes. After extraction of juice from fruit, around 25-30% of the processed fruit is left as waste containing skins and seeds, called as papaya pomace. Generally this goes as a waste causing environmental pollution and if utilized properly will contribute to national economy and reduce pollution effect. Available information on chemical composition and utilization of papaya skins in feeding growing pullets (Fouzder *et al.*, 1999) indicate the potential value of papaya pomace for animal feeding. In view of paucity of information on papaya pomace utilization in animal feeding, an attempt was made to study the effect of inclusion of varying levels of papaya pomace in concentrate mixtures on the nutrient utilization in native male buffaloes.

**Materials and methods** A concentrate mixture with 20% crude protein was prepared by using maize, 270; de-oiled groundnut cake, 300; de-oiled ricebran, 410 and mineral mixture 20 g per kg and was used as control (CM-0). Papaya pomace was included at 10 (CM-10), 20 (CM-20) and 30% (CM-30) levels in experimental concentrate mixtures by adjusting deoiled groundnut cake and deoiled ricebran to make them isonitrogenous. These concentrate mixtures were fed @ 1.35 kg daily along with 4.5 kg of rice straw to meet the nutrient requirements for maintenance as per Kearnl (1982) and were evaluated in a 4x4 latin square design experiment (14 d preliminary period + 7 d collection period ) using four fistulated native male buffaloes (280 ± 2 kg ) to study the nutrient utilization, balances of N, Ca & P and rumen metabolic profiles.

**Results** The feed offered was totally consumed without any refusal and the dry matter intake ranged between 5.35 to 5.36 kg for the four treatments. Inclusion of papaya pomace at 10, 20 and 30% levels in the concentrate mixtures has no significant effect on digestibility of dry matter and crude protein. However, the digestibility of cell wall constituents increased linearly ( $P > 0.05$ ) with the inclusion of papaya pomace in the concentrate mixtures indicating that fibre fraction of papaya pomace was fairly digestible. All the animals were in positive balance for N, Ca and P. The inclusion level of papaya pomace had no significant effect on rumen pH values. However, the NH<sub>3</sub>-N concentration in the rumen liquor of the buffaloes decreased ( $P < 0.05$ , 11.53 to 10.93 mg/100ml of SRL ) and that of TVFA concentration increased ( $P < 0.05$ , 84.77 to 105.61 meq/ L of SRL ) with increase in papaya pomace inclusion in the concentrate mixtures. Perusal of data indicate that pH, NH<sub>3</sub>-N and TVFA concentration were optimal for cellulolytic activity, maximum microbial protein synthesis and for maximum rates of absorption at 30% level inclusion of papaya pomace. The digestible crude protein and digestible energy intakes observed with buffaloes fed on experimental rations were adequate to meet the nutrient requirements as suggested by Kearnl (1982 ) for maintenance of 300kg buffaloes.

**Table 1** Nutrient utilization and plane of nutrition in buffaloes fed concentrate mixtures containing papaya pomace

	CM-0	CM-10	CM-20	CM-30	SEM	STAT.SIG
Dry matter intake (Kg/d )	5.36	5.35	5.35	5.35	0.020	NS
Dry matter digestibility (%)	54.8	56.5	56.1	55.6	1.24	NS
Crude protein digestibility (%)	50.1	57.6	56.8	50.7	1.88	NS
Nuetral detergent fibre digestibility (%)	49.6	51.4	52.0	52.1	1.49	NS
Acid detergent fibre digestibility (%)	43.5	47.3	49.9	46.5	1.65	NS
Hemicellulose digestibility (%)	53.2	55.2	58.1	59.1	4.59	NS
Cellulose digestibility (%)	53.1	54.9	57.7	58.0	2.85	NS
Nitrogen retention (g/d)	7.7	9.2	9.3	8.7	2.00	NS
Calcium retention (g/d)	13.0	15.3	17.4	19.7	1.46	NS
Phosphorous retention (g/d)	19.1	19.1	17.3	16.7	1.93	NS
Digestible crude protein intake (g/d)	211	240	238	211	10.1	NS
Digestible energy intake (MJ/d )	47.3	48.1	48.1	47.3	0.33	NS

NS Non- significant

**Conclusion** It is concluded that papaya pomace can be included at 30% level in concentrate mixtures of native male buffaloes on rice straw based rations for maintenance.

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## Comparative digestive ability and microbial population of Sistani (*Bos indicus*) and Holstein (*Bos taurus*) cattle fed different roughage diets

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**Introduction** There is conflict regarding the relative abilities of *Bos indicus* and *Bos taurus* cattle to digest the feedstuffs. Part of this conflict may have arisen because a wide variety of diets have been fed at different range of intakes and only digestibility in whole tract measured. Generally no attempts have been made to partition rumen and post-rumen digestion or to assess the relative importance of rumen digestion and clearance between diets and between genotypes. Nitrogen (N) is frequently a major limiting nutrient for ruminants specially when they are given diets of mature herbage. On the other hand the extent to which cattle are able to recycle urea-N from blood to rumen for utilization in production of microbial protein could have an important influence on animal survival under condition of severe N limitation. The present experiment was designed to compare rumen microbial population and the end products of rumen fermentation between Sistani (*Bos indicus*) and Holstein (*Bos taurus*) breeds.

**Materials and Methods** Three ruminally fistulated Sistani (S) and three Holstein (H) steers were used in a completely randomized design with factorial arrangement. All animals, in three different periods, fed three types of roughage diet including either alfalfa hay (ALF), *phragmites australis* (PA) or wheat straw (WS) with 149.4, 74.0 and 35.7 g/kg crude protein respectively. The feed offered twice a day (8:00 and 16:00) at about maintenance level of animal requirements. For each feed, data collection was started after 15 days adaptation period. Diurnal changes of pH, concentration of total volatile fatty acids (Kroman *et al* 1967), ammonia nitrogen (Conway 1950) were measured. Rumen degradability of dry matter and crude protein (Orskov and McDonald 1979), and *in vitro* gas production (Menke *et al* 1979) for each feed and two genotype were determined. Total ruminal bacterial population based on most probable number method (Obispo and Dehority 1992) was estimated and concentration of zoospore of anaerobic fungi, total ciliates concentration and *Holotrich* protozoa were determined via direct count method.

**Results** The pH of rumen liquor was not differed between breeds when steers received PA, but by feeding ALF the pH of rumen liquor of Sistani was higher than ( $P < 0.05$ ) Holstein (7.02 vs 6.98 respectively), whereas when they fed with WS, the rumen liquor pH of Holstein was lower than Sistani group. The potential of ruminal total volatile fatty acids production was not significantly different between the two breeds when fed each of the roughages. The concentration of ammonia nitrogen (N-NH<sub>3</sub>) in rumen liquor was similar for two breeds when the animals fed ALF, whereas by consuming PA or WS, the N-NH<sub>3</sub> concentration of Sistani breed was significantly ( $P < 0.05$ ) higher than Holstein (101.63 vs 77.74 and 69.15 vs 53.33 mg/l respectively). There were no significant differences in dry matter and crude protein degradability of ALF, PA or WS when incubated in the rumen of different groups, and the same pattern was observed for total cumulative *in vitro* gas production. The total population of bacteria per ml of rumen liquor was not significantly different between breeds fed different diets. Feeding of PA did not affect the protozoa concentration in the rumen liquor, but when the animals fed ALF or WS the total protozoa and *Holotrich* protozoa number (per ml of rumen liquor) of S were significantly ( $P < 0.05$ ) higher than H group. No significant differences were observed between the groups for the density of ruminal anaerobic fungal zoospore.

**Conclusion** The present results suggest that, the Holstein steers could have better digestive potential and ruminal fermentation of ALF, whereas the Sistani steers may ferment and digest low nitrogen feeds such as PA and WS more efficiently.

**Acknowledgment** we acknowledge the Animal Science Research Institute in Karaj for providing the funds and the facilities required for conducting this project. We wish to thank animal nutrition research department of this institute for support of this work, especially to Dr. M. Zahedifar the head of department for advise and suggestion.

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## The effects of processed whole crop wheat, maize silage and supplement type to whole crop wheat on the performance of dairy cows

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**Introduction** Previous work has shown that processing whole crop wheat (WCW) at harvest increases starch digestibility (Jackson *et al.*, 2002). However, no effect was seen in terms of milk yield. It has been suggested that the provision of a sugar source might utilise the high rumen ammonia levels found in animals receiving urea-treated whole crop wheat (Abdalla *et al.*, 1999). Sources such as lactose have also been shown to reduce rumen protozoa numbers, increase bacterial protein supply and result in a more stable rumen pH, particularly with high starch diets (Hussain and Miller, 1999). Additionally, to date, processed whole crop wheat has not been evaluated against other alternative forages. The objective of the current experiment was, therefore, to compare processed urea treated whole crop wheat with maize silage and determine the effects of carbohydrate supplementation of whole crop wheat on intake and milk production in dairy cows.

**Materials and Methods** A conventionally managed crop of winter wheat (*cv.* Equinox) was harvested on 17 August 2001 and preserved with the aid of a urea + urease product (Home 'n Dry, Volac, Royston, U.K.). A crop of forage maize (*cv.* Nancis) was harvested on 15 October 2001 and preserved with no additive. There were four dietary treatments; WCW supplemented with 2kg rolled wheat (W-WCW), WCW supplemented with 0.7kg/d lactose (Sugarmins, Trouw UK, Northwich) + 1.3 kg/d rolled wheat, (L-WCW), or WCW supplemented with 2.4kg/d cane molasses (M-WCW). An additional treatment (Maize) was maize silage supplemented with 2kg/d rolled wheat and feed grade urea to balance the ration crude protein content. In addition all cows were supplemented with 2kg/d rapeseed meal and 6.5kg/d of dairy concentrate (DM 892g/kg, ME 13.5 MJ/kg DM, and crude protein 249 g/kg DM). The forages were mixed 2:1 (DM basis) with 1<sup>st</sup> cut grass silage. Forty four Holstein-Friesian dairy cows that were on average in week 8 of lactation were allocated to one of the four treatments. Milk yield was recorded daily and samples taken and analysed weekly. Animals were weighed and condition scored weekly and blood sampled upon entering the experiment and at weeks 12, 17 and 22 of lactation. Results were analysed as a randomised block design.

**Results** The WCW had a dry matter (DM) of 823 g/kg, crude protein of 143 g/kg DM and a starch content of 350 g/kg DM. The maize silage had a DM of 310g/kg, crude protein content of 76 g/kg DM, starch of 308 g/kg DM and an ME of 11.5 MJ/kg DM. Supplementation of WCW with molasses (M-WCW) increased DM intake resulting in the highest intake of 23.2 kg DM/d ( $P < 0.01$ ). The lowest DM intake was recorded in animals receiving the Maize treatment (19.9 kg DM/d). Milk yield was not significantly affected by treatment, although there was a trend for animals receiving L-WCW to have a higher milk yield than animals receiving M-WCW. Milk fat content was not affected by treatment but milk protein content was higher ( $P < 0.01$ ) in animals receiving M-WCW. However, there was no effect of treatment on milk fat or protein yield (kg/d). Average liveweight and condition score were also not affected by treatment. Blood urea levels (mmol/l) were lower in animals receiving molasses (M-WCW) ( $P < 0.05$ ) than animals receiving W-WCW or L-WCW.

**Table 1** Effect of processed whole crop wheat, maize silage and supplement type to whole crop wheat on performance

	Maize	W-WCW	L-WCW	M-WCW	s.e.d.	Significance
Dry matter intake (kg/d)	19.9 <sup>a</sup>	22.2 <sup>bc</sup>	21.0 <sup>ab</sup>	23.2 <sup>c</sup>	0.88	**
Milk yield (kg/d)	34.0	34.4	35.6	33.1	1.18	NS
Fat (g/kg)	37.7	34.3	34.3	38.4	2.20	NS
Protein (g/kg)	31.2 <sup>a</sup>	32.5 <sup>ab</sup>	31.5 <sup>a</sup>	33.3 <sup>b</sup>	0.61	**
Fat yield (kg/d)	1.31	1.18	1.22	1.27	0.075	NS
Protein yield (kg/d)	1.07	1.13	1.11	1.10	0.040	NS
Liveweight (kg)	585	613	585	606	21.6	NS
Condition score	2.53	2.71	2.60	2.50	0.161	NS
Plasma urea (mmol/l)	5.76 <sup>a</sup>	5.87 <sup>a</sup>	5.81 <sup>a</sup>	5.06 <sup>b</sup>	0.260	*

**Conclusions** Feeding processed WCW results in a similar level of performance to that of maize silage. Supplement type had no significant effect on milk yield, but supplementation with lactose tended to increase milk yield. Feeding molasses significantly increased milk protein content but not milk protein yield.

**Acknowledgements** The project was funded by the Milk Development Council and the Maize Growers Association.

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# Ruminal and intestinal protein disappearance of some tropical (Iranian) feeds used in dairy cow diets estimated by the mobile nylon bag technique

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**Introduction** In Iran milk production, in dairy cows, is based mainly on intensive systems in which cereals and industrial by-product are the most important source of nutrients. So, high digestible protein feeds are often desired for supplementation of high producing dairy cows to meet their amino acid requirements. Incubation of feeds in nylon bags in the rumen of fistulated ruminants have been used to evaluate the extent of digestion. In addition, the mobile bag technique has been used to measure intestinal digestibility of undegraded dietary protein and intact feed protein (Mesgaran, 2002). The work described in this summary assessed the digestibility of dry matter and protein, using ruminal and intestinal mobile nylon bag technique, of some tropical (Iranian) feeds used in dairy cow diets.

**Materials and methods** The ruminal, post ruminal and total tract disappearance of dry matter and protein of samples were determined using the mobile nylon bag procedure (Mesgaran, 2002). The experimental feeds were barley, maize, wheat bran, sugar beet pulp, lucerne hay and silage, maize silage, whole barely silage, wheat straw, soybean and cottonseed meals, and fish meal. The plant feeds originated from the Iranian varieties and fish meal from a variety located in Caspian Sea. Four Holstein steers (395±13 kg) fitted with rumen fistula and T-shaped cannulae were used in the present study. They fed 5.1 Kg DM of good quality lucerne hay, 1.2 Kg DM maize silage and 2.7 Kg DM concentrate (171 g CP Kg<sup>-1</sup> DM) per steer per day. The bags (3x6 cm) were made of Dacron cloth with a pore size of 46 µm. About 1.2 g dry matter of each feed (grounded through 2 mm screen) was placed in each bag (16 bags per each feed), then inserted into plastic mesh cylinders (26x8 cm, 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 post ruminal digestibility (8 bags per each protein source) were then inserted into the small intestine via the cannulae at the rate of one bag every 30 min and removed from the voided faeces, rinsed in cold running 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. Data were presented as Mean±SD.

**Results** The data related to the disappearance of DM and protein from mobile bags within rumen, intestine and total tract are shown in Table 1. The results indicated that the disappearance values for DM and protein were lower for forages than for cereals and protein sources.

**Table 1** Dry matter and protein disappearance (g g<sup>-1</sup>) of some tropical (Iranian) feeds, used in dairy cow diets, from mobile nylon bags during incubation in the rumen and passage through the intestine

Feed	Intact feed -disappearance in the rumen		Rumen undegraded - disappearance in the intestine		Intact feed - disappearance in total tract	
	DM	Protein	DM	Protein	DM	Protein
Barely grain	0.63±0.05	0.56±0.04	0.66±0.06	0.61±0.05	0.87±0.06	0.83±0.05
Maize grain	0.35±0.06	0.31±0.04	0.61±0.07	0.53±0.04	0.77±0.07	0.68±0.04
Sugar beet pulp	0.59±0.07	0.61±0.05	0.59±0.03	0.52±0.03	0.85±0.07	0.82±0.04
Maize silage	0.35±0.03	0.37±0.06	0.58±0.03	0.49±0.04	0.73±0.03	0.68±0.05
Whole barley silage	0.54±0.05	0.61±0.05	0.50±0.04	0.42±0.03	0.77±0.05	0.78±0.05
Lucerne hay	0.44±0.04	0.55±0.05	0.53±0.07	0.53±0.04	0.74±0.06	0.79±0.05
Lucerne silage	0.57±0.04	0.60±0.05	0.55±0.04	0.62±0.05	0.78±0.04	0.83±0.04
Wheat straw	0.19±0.03	0.16±0.04	0.16±0.03	0.15±0.03	0.32±0.03	0.30±0.03
Wheat bran	0.56±0.04	0.61±0.04	0.49±0.04	0.52±0.03	0.78±0.04	0.82±0.03
Soybean meal	0.48±0.06	0.59±0.04	0.68±0.03	0.66±0.04	0.84±0.04	0.87±0.04
Cottonseed meal	0.31±0.02	0.42±0.05	0.60±0.04	0.61±0.05	0.73±0.03	0.78±0.05
Fish meal	0.33±0.03	0.31±0.02	0.64±0.05	0.66±0.04	0.78±0.04	0.77±0.03

**Conclusion** The results suggest that the ruminal protein disappearance of the forages, evaluated in this study, is of great importance for estimating the protein value of these feeds. The ruminal and post-ruminal DM and protein disappearances of protein sources were notably higher compared to those of energy and forage sources. These data agreed with the finding of the other workers and may be due to the chemical condition of these feeds. It has also been demonstrated that most plant proteins are predominantly digestible in ruminants, though could supply appreciable quantities of digestible protein in dairy cows.

**Acknowledgement** The author wishes to acknowledge from the Ferdowsi University of Mashhad, Iran, for funding this project.

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## Supplementation of finishing Nellore steers during dry season using byproducts

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**Introduction** Beef cattle production based in tropical pasture has a period of low daily weight gains during dry seasons, because the lowest quality and quantity pastures in this period. This study evaluated the effects of protein supplementation in animal performance and carcass characteristics of Nellore cattle on tropical pasture.

**Material and Methods** A hundred and twenty eight animals had been evaluated at IZ/Nova Odessa-São Paulo-Brazil 22 lat. and 47 long. The animals were 400 kg initial average weight and 2 years old at the beginning of the experiment. The statistical design was a randomized block with four treatments and four replications. Least square means test was done. The treatments were: Control = *Brachiaria brizantha* pasture + mineral supplement; Leucena = *Brachiaria brizantha* pasture + protein bank (*Leucaena leucocephala*) + 2 kg citrus pulp + 2 kg Wet corn gluten feed + mineral supplement (animal/day); Supl. 1 = *Brachiaria brizantha* pasture + 2 kg citrus pulp + 2 kg Wet corn gluten feed + 0.5 kg soybean meal + mineral supplement (animal/day); Supl. 2 = *Brachiaria brizantha* pasture + 2 kg citrus pulp + 2 kg Wet corn gluten feed + 1 kg soybean meal + mineral supplement (animal/day). The animals were weighted by 28 days and backfat measures were made on the right carcasses after 24 hours colding.

**Results** The supplementation provides higher daily weight gain to the supplemented animals. The average daily gains were 0.114, 0.528, 0.638 and 0.613 kg/animal/day for control, leucena, Supl. 1 e 2, respectively (table 1). Our results were similar to Ruas, et al., (2000) and Bandyk et al., (2001). It was observed higher carcass yield for the supplemented animals of the treatments leucena, Supl. 1 e 2. The backfat values had the same behavior of yield carcass, but when the backfat values were related to 100 kg of carcass, no difference was observed (table 2).

**Conclusion** The protein supplementation provides higher daily weight gain and reduction the age of slaughter, because increases in growth velocity. In addition to this, the treatments leucena, Supl. 1 e 2 had higher carcass yield and backfat values, which provided an increase in quality of carcass retail. The supplementation of finishing steers on tropical pasture is an efficient practice to increase the animal production and beef quality.

**Table 1** Average daily gains for different treatments of total experimental period.

Treatments	kg/animal/day
Control	0.114 <sup>a</sup> ± 0.051
Leucena	0.528 <sup>b</sup> ± 0.042
Supl.1	0.638 <sup>c</sup> ± 0.035
Supl.2	0.613 <sup>c</sup> ± 0.031

\* means with unlike superscripts are different (P ≤ 0.05)

**Table 2** Backfat for different treatments.

Treatments	Backfat/100 kg carcass (mm)	Backfat (mm)
Controle	1.34 <sup>a</sup> ± 0.08	3.30 <sup>a</sup> ± 0.20
Leucena	1.56 <sup>a</sup> ± 0.10	4.24 <sup>ab</sup> ± 0.31
Supl.1	1.73 <sup>a</sup> ± 0.12	4.75 <sup>b</sup> ± 0.33
Supl.2	1.76 <sup>a</sup> ± 0.14	4.87 <sup>b</sup> ± 0.40

\* means with unlike superscripts are different (P ≤ 0.05)

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## Effect of energy source and supplementation pattern on feed intake and microbial-N supply in dual purpose cows (*Bos indicus* x *B. taurus*)

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**Introduction** The microbial protein synthesis is variable and depend of several of factors, one the most important being the energy sources. Numerous investigations have evaluated individual sugars. However, few quantitative information is available describing the impact of sucrose or its monosacharides (glucose and fructose) and how they compare with other carbohydrate such as starch (Heldt *et al.*, 1999). Chamberlain and Choung (1995) mention that sugar supplementation results in a higher microbial nitrogen supply (MNS) than starch. On the other hand, the amount of nutrients that ruminants can remove from feeds, may be modified by others feeds eaten the same day (Gill and Powell, 1993). Thus, feeding pattern might also affect microbial-N supply (Chamberlain and Choung, 1995). Therefore, the objective of the present work was to compare maize and sugarcane molasses as energy sources as well as the change of feeding pattern (CFP) on DM intake and MNS.

**Material and methods** Five crossbreed cows (*Bos indicus* x *B. taurus*) (362 ± 28.71Kg LW) fitted with rumen fistulas, were used. Treatments were: 1. Two energy sources (sugar-cane molasses and maize) (E) and a control without energy supplementation (NE); 2. Two feeding times (AM and PM). The animals had *Pennisetum purpureum* (chopped) fed at 120 % of the previous day intake as basal diet and also received one kg DM of a foliage mix (0.37:0.63) of *Brosimum alicastrum*:*Leucaena leucocephala* as protein supplementation. Cows were housed in metabolic crate with *ad libitum* access to water. Forage was accessible from 18:00 to 06:00 h. The supplements were given at 09:00 and 17:00 h for AM and PM respectively. The trial lasted 13d (7d adaptation and 6d measurements) where voluntary intake, pH, NH<sub>3</sub>-N (rumen fluid samples were taken at 0, 1, 2, 3 and 7 h after the supplement was fed) and purine derivatives in urine (total collection) were measured daily. The purine derivatives excretion were converted to microbial nitrogen as suggested by Verbic *et al.* (1990). Data were analyzed using the GLM procedure of Statgraphics Plus for Windows (1999) with a model appropriate for a 5 x 4 incomplete Latin square with a 2 x 3 factorial arrangement. Orthogonal contrasts were used to compare 1)NE vs. E, 2) molasses vs. maize and 3) morning vs. evening feeding.

**Table 1.** Chemical composition (g/kgDM except where stated)of basal diet and supplements

	DM <sup>3</sup>	OM	CP	NDF	ADF
<i>P. purpureum</i>	950	902	53	777	516
Foliage mix <sup>1</sup>	926	816	210	444	265
Maize	909	895	94	142	21
Molasses <sup>2</sup>	743	610	-	-	-

1:(0.37:0.63) of *B. alicastrum* : *L. leucocephala*,  
2: Sugarcane molasses, 3: g/kg fresh base

**Table 2.** Intake (kg/d) and microbial-N supply (MNS g/d) and efficiency of MN synthesis (gMN/kg DOMR) with (E) and without (NE) an energy supplement (sugarcane mollases (Mo) or maize (Ma)) fed after (AM) or before (PM) the basal diet (grass)

	NEAM	MoAM	MaAM	NEPM	MoPM	MaPM	1	2	3
<i>P. purpureum</i>	5.6±0.16a	5.4±0.2a	5.8±0.2a	5.7±0.2a	5.2±0.2a	5.5±0.16a	ns	ns	ns
Total DM	6.6±0.16b	7.6±0.2a	7.9±0.2a	6.7±0.2b	7.4±0.2a	7.5±0.16a	ns	*	ns
Total OM	5.9±0.15b	6.7±0.18a	6.8±0.18a	5.6±0.18b	6.6±0.18a	6.8±0.15a	ns	*	ns
Allantoin	5.6±0.69b	8.7±0.85ac	6.9±0.86ab	5.0±0.85b	10.3±0.86c	6.9±0.69ab	-	-	-
Uric acid	2.2±0.38b	3.7±0.46a	2.2±0.47b	1.82±0.46b	4.0±0.47a	2.3±0.38b	-	-	-
MNS	27.8±3.55b	47.3±4.34a	33.7±4.37b	23.9±4.34b	55.8±4.37a	33.6±3.55b	ns	*	*
Efficiency MN	9.8±1.18a	15.0±1.44b	9.3±1.45a	9.4±1.44a	16.7±1.45b	9.85±1.18a	ns	ns	*

1: AMvsPM, 2: EvsNE, 3: MovsMa, values after ± are s.e.m., Means with different literal in the same line differ at P<0.05

**Results** Chemical composition of the feeds are presented in table 1. There were not significant (P > 0.05) differences on basal diet intake (Table 2). pH means were above 7 in all treatments Ammonia concentrations were within appropriated ranges (20 – 50 mg/l). MNS was increased (P<0.05) with E compared with NE (43 vs. 26 g/d), but molasses resulted in a higher (P<0.05) MNS than maize (51.45 vs. 33.67 g/d). Also MNS with molasses was more efficient (P < 0.05) than with maize (15.82 vs. 9.64 g MN/kg DOMR).

**Conclusion** Changes in feeding pattern did not affect neither MNS nor efficiency of MN synthesis. However, energy supplementation increased MNS, especially molasses, which also improved the efficiency of MN synthesis.

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## Concentrate restriction and its substitution by liquid whey in feeding of Holstein steers

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**Introduction** A large quantity of whey is produced in Iran and mostly is discharged into streams and seasonal rivers, which cause environmental pollution. Whey as a nutritious dairy by-product can effectively be fed to ruminants (Bayat *et al.* 2002). This experiment was conducted to study the effects of concentrate substitution by liquid whey on performance, rumen and blood parameters of the Holstein steers.

**Materials and Methods** Twelve Holstein steers with average body weight of 150 kg (s.d. 27) were selected and stratified based on weight into 3 groups and randomly assigned to the treatments. Experimental design was Completely Randomized Design with three treatments and four replications. Lucerne hay was fed at the level of 0.7 percent (DM Basis) of body weight. Liquid whey was given ad lib. No drinking water was provided. Experimental treatments were: I. Normal concentrate feeding (ad lib) as control, II. Concentrate restricted at the level of two third of concentrate intake of the control, III. Concentrate restricted at the level of one third of concentrate intake of the control. The experimental period was 100 days including 15 days adaptation and 85 days sample collection period. Lucerne hay and concentrate were fed separately. Diet was formulated based on NRC (1989) recommendations for 3-6 month growing calves. Blood samples from jugular vein were taken 3h after morning feeding and immediately centrifuged (3000 rpm and 10 minutes) to obtain their plasma. Rumen liquor samples were taken 3h after morning feeding by stomach tube and evacuation pump. Analyses of feed and feces were conducted based on AOAC (1984). Analyses of NDF and ADF were conducted based of Van Soest method (Georging and Van Soest 1970). Acid insoluble ash (AIA) was used as internal marker for apparent digestibility determination (Van Keulen and Young 1977). Data were analyzed by SAS software (1996).

**Results** Whey consumption of treatment III increased by 12.68 percent in comparison to treatments I and II (Table1). The steers in treatments I, II and III obtained 41.2, 49.3 and 55.7 percent of their daily dry matter intake (DMI) from whey respectively. There was a significant difference among total DMI. The lowest and highest intakes were observed in treatments III and I respectively. Lucerne intake in treatment III was higher than treatments I and II ( $P<0.05$ ).

**Table 1** Feed intakes of the steers (kg/d)

	Treatment			SEM
	I	II	III	
Whey				
As Fed	48.4	48.5	54.64	4.125
DM	2.63	2.63	2.96	0.224
Lucerne (DM)	1.11	1.22	1.45	0.067
Concentrate(DM)	2.64	1.58	0.90	0.139
Total DMI	6.38	5.43	5.31	0.118

**Table 2** Nutrient apparent digestibilities (g/g)

	Treatment			SEM
	I	II	III	
DM	0.76	0.82	0.87	0.034
OM	0.77	0.83	0.88	0.032
CP	0.80	0.85	0.91	0.030
CF	0.34	0.52	0.63	0.090
NDF	0.41	0.58	0.64	0.086
ADF	0.24	0.49	0.64	0.082

Among the nutrient apparent digestibilities (Table 2) only apparent digestibility of ADF in treatment III was significantly increased in comparison to the control ( $P<0.05$ ). Average daily weight gain (ADG) and feed conversion ratio (FCR) are shown in Table 3. Increasing concentrate restriction leads to linear decrease in ADG although it was not significant. There was no significant difference among feed conversion ratios obtained in this study. Rumen and blood

**Table 3** Average daily gain and feed conversion ratio.

	Treatment			SE
	I	II	III	
ADG (kg/d)	1.415	1.247	1.103	0.096
FCR (kg/kg)	4.56	4.42	5.26	0.410

pH and ammonia nitrogen ( $\text{NH}_3\text{-N}$ ) were similar among the treatments. Plasma urea nitrogen (PUN) in treatments II and III were significantly ( $P<0.05$ ) less than the control (14.87 and 18.60 vs. 25.15 mg/dl, SE = 1.637) ( $P<0.05$ ).

**Conclusions** It can be concluded from the results of this experiment that substitution of concentrate by liquid whey in an appropriate ratio could be efficient and economical feeding strategy in the area. Although this conclusion is highly attributed to the market price of feed ingredients.

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# Effect of 9,10-Anthraquinone on rumen methane production as studied *in vitro* and *in vivo*

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**Introduction** *In vitro* supplementation of 0.05 % [on a substrate basis (wt/wt)] - but not of 0.01 % - of 9,10-Anthraquinone (AQ) inhibited rumen methanogenesis, reduced total volatile fatty acid (VFA) concentrations and molar proportions of acetate (Acet), increased proportions of propionate (Prop) and butyrate (But) and resulted sometimes in H<sub>2</sub> accumulation (Garcia-Lopez *et al.*, 1996). *In vivo* administration of high amounts of AQ [5 % on a substrate basis (wt/wt)] to lambs depressed CH<sub>4</sub> and increased H<sub>2</sub> concentrations in ruminal gases during the complete 19 days of administration, whereas original concentrations were re-installed within 6 days after the removal of AQ from the diet (Kung *et al.*, 1996). In this experiment we aimed to study the dose effect of AQ on *in vitro* rumen fermentation and modifications to rumen fermentation when administering 0.05 % of AQ *in vivo*.

**Materials and methods.** *In vitro*, 450 mg of hay were incubated with 50 mg of a mixture of pure wheat middlings/wheat middlings containing 0.5 % AQ in ratios of 0/1; 1/0; 1/1 and 3/1, w/w on a DM basis equivalent to 0, 0.05, 0.025 and 0.0125 % AQ in the substrate. Mixed rumen contents were obtained before the morning feeding from two rumen fistulated sheep, fed twice daily at maintenance a hay/grain based concentrate (65/35, w/w DM) diet. Contents were filtered through a 1 mm sieve and incubations and statistics were carried out as described by Fievez *et al.* (2001). The *in vivo* experiment covered three subsequent periods (PI, PII, PIII) of 12 days each, in which 2 wethers were daily fed 4 portions of 250 g DM hay, 80 g DM grain based concentrate and 3.3 g wheat middlings, pure (PI, PIII) or containing 0.5 % AQ (PII). Sampling, analyses and statistics were as described by Mbanzamihiho *et al.* (2002). In PI and PIII, rumen CH<sub>4</sub> production and VFA concentrations were measured on day 4, 8 and 12; in PII an extra measurement on the first day of AQ supplementation was added.

**Results** *In vitro* AQ administration provokes a dose dependent CH<sub>4</sub> inhibition, accompanied by a decrease in Acet and (tendency of) increased Prop and But production and H<sub>2</sub> accumulation (Table 1). Total VFA production was not inhibited by AQ (data not shown). *In vitro* AQ administration presumably stimulates accumulation of other intermediate products, besides H<sub>2</sub>, as indicated by the negative correlation between CH<sub>4</sub> inhibition and hydrogen recoveries [(2Prop+2But+4CH<sub>4</sub>+H<sub>2</sub>)/(2Acet+Prop+4But)] ( $r_{\text{pearson}} = -0.94$ ,  $p < 0.001$ ,  $n = 18$ ). *In vivo* administration of AQ [0.05 % on a substrate basis (wt/wt)] depressed rumen CH<sub>4</sub> productions and Acet concentrations, whereas Prop and But proportions were increased (Table 2). However, the increase in Prop proportion and reduction in CH<sub>4</sub> production disappears within 8 days after starting the AQ administration, whereas changes in Acet and But were significant during the whole period of AQ administration, possibly through a promotion of the production of But from Acet by AQ. No H<sub>2</sub> was detected in ruminal gases (data not shown). After stopping the AQ administration (PIII) the fermentation pattern (CH<sub>4</sub> production, proportions of individual VFA, total VFA concentration) returned to its original situation.

**Table 1** Dose effect of AQ on relative *in vitro* (24h) rumen CH<sub>4</sub>, VFA and H<sub>2</sub> production (mmol/mol total VFA) and hydrogen recovery (2HR, %) (mean, n = 6)

% AQ	0	0.0125	0.025	0.05	s.e.m.	P-value
CH <sub>4</sub>	297 <sup>a</sup>	232 <sup>ab</sup>	230 <sup>ab</sup>	177 <sup>b</sup>	13.8	*
Acet	578 <sup>a</sup>	562 <sup>ab</sup>	534 <sup>b</sup>	492 <sup>a</sup>	8.1	***
Prop	187	197	203	206	4.3	0.425
But	134 <sup>a</sup>	125 <sup>a</sup>	141 <sup>a</sup>	160 <sup>b</sup>	3.8	**
H <sub>2</sub>	1.5	2.9	30.9	44.8	10.0	0.352
2Hr	97.4 <sup>a</sup>	86.4 <sup>ab</sup>	89.4 <sup>ab</sup>	81.3 <sup>b</sup>	2.3	0.075

**Table 2** *In vivo* effect of AQ administration on rumen CH<sub>4</sub> production (l/d), total (mmol/l) and individual VFA concentrations (mmol/mol total VFA) [mean (sdev)]

	n	CH <sub>4</sub>	Acet	Prop	But	VFA
PI	6	23.7 <sup>a(2.0)</sup>	709 <sup>a(17)</sup>	153 <sup>b(9)</sup>	106 <sup>ab(16)</sup>	78 <sup>ab(10)</sup>
PII/1 <sup>(1)</sup>	2	20.2 <sup>ab(3.3)</sup>	677 <sup>b(17)</sup>	170 <sup>a(9)</sup>	117 <sup>ab(15)</sup>	68 <sup>ab(22)</sup>
PII/4 <sup>(1)</sup>	2	16.3 <sup>b(0.3)</sup>	670 <sup>b(15)</sup>	171 <sup>a(12)</sup>	119 <sup>ab(9)</sup>	69 <sup>ab(5)</sup>
PII/8 <sup>(1)</sup>	2	22.0 <sup>a(2.4)</sup>	668 <sup>b(17)</sup>	169 <sup>ab(10)</sup>	121 <sup>a(21)</sup>	86 <sup>a(23)</sup>
PII/12 <sup>(1)</sup>	2	20.9 <sup>ab(3.5)</sup>	680 <sup>b(36)</sup>	162 <sup>ab(14)</sup>	112 <sup>ab(37)</sup>	54 <sup>b(11)</sup>
PIII	6	24.1 <sup>a(3.2)</sup>	705 <sup>a(39)</sup>	156 <sup>b(8)</sup>	101 <sup>b(32)</sup>	87 <sup>a(21)</sup>
P-value		*	***	*	*	*

PI, PII, PIII period without AQ, with AQ and without AQ (after period with AQ administration), respectively. <sup>(1)</sup> number right of slash indicates number of days after start of AQ administration.

**Conclusions** Adaptation of rumen metabolism to AQ limits scope for the use of AQ as CH<sub>4</sub> inhibitor.

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# Effect of imbalance between energy and nitrogen supplies on microbial protein synthesis in growing double-musced Belgian Blue bulls

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**Introduction** Balancing the supply of nitrogen and energy-yielding substrates to rumen micro-organisms was proposed as a mechanism to maximise the capture of rumen degradable nitrogen (RDN) and to optimise microbial growth rate and efficiency. The objective of this study was to examine the effect of various time periods of imbalance between nitrogen and energy supplies for the rumen micro-organisms on the microbial protein synthesis (SPM) in growing double-musced Belgian Blue bulls. This was realised by giving the same feedstuffs according to different meal patterns, which is one of the most robust test of the 'synchrony' hypothesis (Dewhurst *et al.*, 2000).

**Materials and methods** Six double-musced Belgian Blue bulls initially weighing  $345 \pm 16$  kg and fitted with a ruminal cannula and a T-type cannula at the proximal duodenum were used in the study. The bulls received the same diet according to three different feeding patterns, so that three different time periods of imbalance between energy and nitrogen supplies for the rumen microbes were created. The diet gave 97.5 g of intestinal digestible proteins (DVE) and 1842 kcal of NEF per kilogram of DMI according to the Dutch system. Feed was provided twice a day in equal amounts at 0830 and 2030 at an intake level of 85 g DM/kg<sup>0.75</sup>. The first feeding pattern of the diet (0h) consisted in giving at each meal all the feed ingredients, which resulted in a RDN:FOM ratio equal to 24 g/kg of fermentable organic matter (FOM). Therefore, the energy and nitrogen-yielding substrates to the rumen micro-organisms were considered synchronised. The second pattern (12h) consisted in giving at the morning meal the feed ingredients, which primarily provided energy for the rumen microbes (ENE) and at the evening meal the feed ingredients, which primarily provided nitrogen (PRO). An imbalance of a duration of 12 h between the energy and nitrogen supplies for the rumen microbes was thus experimentally created. The RDN:FOM ratios amounted to 19 and 29 g/kg, for ENE and PRO respectively. However, the RDN:FOM ratio per day was 24 g/kg and the same feedstuffs were ingested. The last feeding pattern (24h) consisted in increasing the time period of imbalance to 24 h by feeding alternately ENE at the two meals of a day and PRO at the two meals of the day after. The RDN:FOM ratio per 48 h was also 24 g/kg and the same amounts of each feed ingredients were ingested compared 0h and 12h. The bulls were allocated to three treatment periods in two juxtaposed 3×3 Latin squares. Rumen fermentation was monitored by pH and ammonia concentration. Total digesta and microbial protein flows to the proximal duodenum were measured using chromic oxide as an indigestible flow marker and purines as a microbial marker.

**Results** No significant differences in rumen pH were seen between the different feeding patterns of the diet. On the other hand, the ruminal ammonia concentration was highly influenced by the nature of the feed ingredients ingested (ENE, PRO or both) and attested the existence of periods of excess and deficiency in N supply. The period of time that rumen ammonia concentration was below 5 mg/dl depended on the meal ingested and lasted on average, respectively for ENE, PRO and both simultaneously, 9h, 0h and 6h over 12h. Duodenal dry matter, organic matter and total nitrogen flows (Table 1) were similar among the feeding patterns. The duration of imbalance did not affect the organic matter apparently digested in the rumen (OMADR). Microbial nitrogen flows to the duodenum, expressed in g/d or in % Non ammonia-N (NAN), were not significantly different. The efficiencies of rumen microbial protein synthesis (ESPM) were not affected by the duration of imbalance between the energy and nitrogen supplies and reached on average 29 g N/kg OMADR. These observations supported the hypothesis proposed by Dawson (1999) that the ruminants and their microbes can detect asynchrony in the rate of nutrient supply and have developed mechanisms to overcome or minimise its effects.

**Table 1** Nitrogen intake, nitrogen components flowing to the duodenum and efficiency of microbial protein synthesis (ESPM).

	0h	12h	24h	SEM	p
N intake (g/d)	170.1	173.8	172.0	1.2	0.17
Duodenal flow (g/d):					
- Total N	172.8	181.3	179.6	5.6	0.55
- Non NH <sub>3</sub> -N (NAN)	168.5	176.9	174.8	5.3	0.54
- Microbial-N	70.1	73.6	72.6	2.8	0.69
Microbial-N/ NAN (%)	41.7	41.6	41.5	0.8	0.98
ESPM (g N/kg OMADR)	26.7	31.2	29.1	2.6	0.50

similar among the feeding patterns. The duration of imbalance did not affect the organic matter apparently digested in the rumen (OMADR). Microbial nitrogen flows to the duodenum, expressed in g/d or in % Non ammonia-N (NAN), were not significantly different. The efficiencies of rumen microbial protein synthesis (ESPM) were not affected by the duration of imbalance between the energy and nitrogen supplies and reached on average 29 g N/kg OMADR. These observations supported the hypothesis proposed by Dawson (1999) that the ruminants and their microbes can detect asynchrony in the rate of nutrient supply and have developed mechanisms to overcome or minimise its effects.

**Conclusions** The introduction of an imbalance of 12 or 24 hours between the energy and nitrogen supplies for the rumen micro-organisms by changing the feeding pattern of the same feedstuffs, did not affect the different nitrogen components flowing to the duodenum or the efficiency of microbial protein synthesis. It would appear that a lack of instantaneous synchronisation between the energy and nitrogen supplied for the rumen microbes is not prejudicial to their growth as long as the nutrient supply is balanced on a 24 or 48 hours basis.

**Acknowledgements** The research was funded by FRIA, Brussels.

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## Winter feeding regimes for 16-22 month old red deer stags and hinds

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**Introduction** Farmed red deer (*Cervus elaphus*) are highly seasonal animals and thus the majority of venison is available during the autumn and winter months. To compete with other red meats, alternative marketing systems for venison need to be developed. Previous ADAS trials have shown that red deer can be finished intensively at 10-12 months of age by extending day length (Davies *et al*, 1995) but it incurs increased feed costs. An alternative strategy to lower production costs is to reduce growth rates and finish deer at 24-26 months of age. Preliminary trials have shown (Davies and Deakin 1998) that silage only diets, during the second winter, are not sufficient to maintain body weight and a small supplement is required. However the type of supplement needs further investigation. The objective of this study was to examine the effect of feeding high energy and protein diets to yearling deer during their second winter, on winter growth and subsequent performance at grass.

**Materials and methods** Seventy-two yearling deer (36 stags and 36 hinds) were housed on 31 October 2000 and fed one of three diets over the winter period. They were fed either *ad libitum* grass silage plus 0.3 kg barley/head/day (SB), restricted grass silage plus 0.8 kg/head/day high fat (DM 859 g/kg, ME 13.5 MJ/kg, CP 187 g/kg) compound diet (HF) or restricted grass silage plus 0.8 kg/head/day high protein silage (DM 856 g/kg, ME 13.0 MJ/kg, CP 245 g/kg) compound diet (HP). There were two pens of six deer on each treatment for both stags and hinds. The grass silage (DM 251 g/kg, ME 10.6 MJ/kg, CP 153 g/kg) was fed once a day and the barley (DM 884 g/kg, ME 13.2 MJ/kg, CP 123 g/kg) and compound was fed on top of the silage. The SB deer were only fed barley from 3 January to turnout. Daily ME intakes were 13.0, 15.6 and 15.6 MJ/kg for stags and 8.8, 13.6 and 13.8 MJ/kg for hinds fed SB, HF and HP respectively. The deer were turned out to grass on 3 May and the winter feeding period was 182 days. During the summer, the deer grazed as one group on perennial ryegrass/white clover swards maintained at 8-10 cm sward height. Live weight data were analysed by analysis of variance.

**Results** All three treatments of stags and hinds lost a similar amount of live weight in early winter (mean -26 g/day for stags and -47 g/day for hinds). In late winter, recovery of early live weight loss on HF and HP diets were significantly higher ( $P < 0.001$ ) than on SB diets. Overall winter growth rates were significantly higher ( $P < 0.01$ ) on HF and HP diets. Summer growth rates were inversely related to winter gain. However, full compensation did not occur and deer fed SB winter diets were finished at lighter weights.

**Table 1** Daily live weight gain (g) of yearling red deer hinds and stags

Treatment	Stags					Hinds				
	SB	HF	HP	s.e.d	sig.	SB	HF	HP	s.e.d	sig.
Live weight (kg):										
Housing	83.5	83.6	83.5	0.94	NS	72.4	72.5	72.3	0.63	NS
Turnout	85.7	94.0	94.8	3.42	*	63.2	75.5	77.4	2.35	***
July	104.3	113.8	110.0	4.38	NS	75.1	81.0	82.1	3.04	NS
Daily gain (g):										
Early winter	-33	-15	-30	31.8	NS	-78	-24	-38	22.4	NS
Late winter	12	88	100	18.8	***	-38	39	48	13.5	***
Overall winter	6	58	61	17.3	**	-51	17	24	11.1	***
At grass	242	258	197	32.9	NS	211	128	99	36.7	*

**Conclusions** Feeding yearling red deer stags and hinds for live weight maintenance reduce costs substantially and allows compensatory growth at pasture. However, when 16-22 month old deer are fed *ad libitum* silage with a small supplement, intakes are inadequate and hinds lose weight. Feeding a high fat or high protein diet to achieve energy levels of 16 and 13 MJ/kg/day for stags and hinds respectively will maintain deer through the winter and produce higher weights at slaughter.

**Acknowledgements** This work was funded by the Department of Environment, Food and Rural Affairs.

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## Production response to increased calcium salts of palm fatty acids in dairy cows

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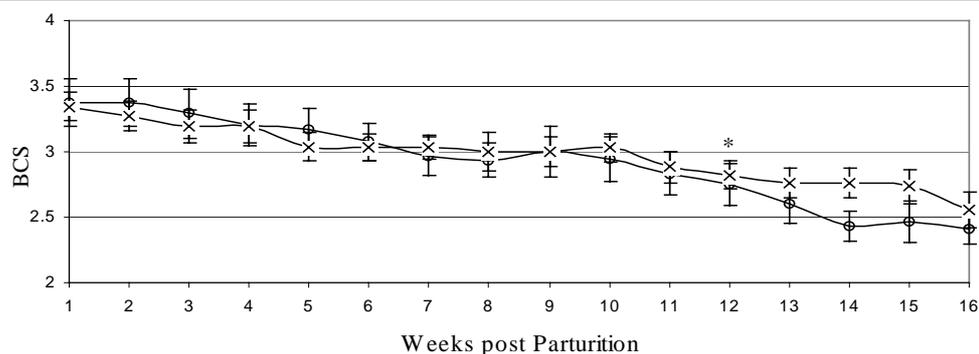
**Introduction** High levels of dietary concentrates are often used to support milk production and it is important to investigate ways to feed them efficiently. Fats have the greatest energy density of any feed ingredient and the inclusion of protected fats in the dairy cow ration enables a high energy but balanced ration to be fed. The aim of this trial was to investigate the effect of feeding different levels of Megalac (Volac International Ltd, Herts) to dairy cows and to compare milk yield, milk composition, fertility and body condition score (BCS).

**Materials and Methods** Thirty-two non-lactating pregnant dairy cows were paired according to previous lactation yield, £PIN value and parity. At parturition one cow from each pair was randomly assigned to one of two treatment groups (LR and HR). Low rate cows were fed on Premium Winter dairy concentrate (Pye Farm Feeds, Lancaster) containing maize, maize based products, rapemeal, field beans, wheat & barley, molasses, breakfast cereal and 20g/kg of Megalac (calcium soaps of palm fatty acids). High rate cows received the same concentrate to which 60g/kg Megalac had been added. Cows were recognised by transponder in the parlour and the appropriate diet was dispensed to yield at each milking for 16 weeks postpartum. The cows received an identical TMR *ad libitum* of grass, maize, whole crop wheat and whole crop pea silage dispensed by a mixer wagon. Cows were given access to pasture at week 12. Average weekly milk yields were recorded and milk protein and milk fat concentrations were measured fortnightly and fertility data was collected. Cows were condition scored on a weekly basis using Mulvaney's 5 point scale. Interpretation using analyses of variance on the milk yields and milk composition involved using the previous yield day 305 figure as a co-variate.

**Results** There was a tendency over the first 16 weeks postpartum ( $P=0.075$ ) for the milk yield in the HR treatment group to be increased by 2.37kg/d compared to the LR treatment group, see Table 1. Milk yields of weeks 1-8 did show a statistically significant difference,  $P<0.05$  between the two treatment groups. There was no significant difference in the protein and fat content of the milk or in the somatic cell count. The fertility data showed no significant differences in days to first service, number of services, days open and pregnancy rate between the two groups. There was no difference in BCS, see Figure 1, between the two groups until turn-out when the LR group lost, and the HR group maintained, body condition. This difference was statistically significant, ( $P<0.05$ ) from week 12 onwards.

**Table 1** Effect of increased Megalac on milk yield milk composition and fertility in the thirty-two paired cows

	Megalac (LR)	Megalac (HR)	s.e.	<i>P</i>
Milk yield wk 1-16 (kg/d)	32.21	34.58	1.68	0.075
Milk yield wk 1-8 (kg/d)	31.23	33.49	1.75	0.022
Milk yield wk 9-16 (kg/d)	33.19	35.68	1.62	0.312
Milk fat (g/kg)	3.87	3.96	0.06	0.560
Milk protein (g/kg)	3.24	3.24	0.05	0.552
Somatic cell count (1000 cells/ml)	279.21	206.42	109.38	0.292



**Figure 1** Body condition scores of the LR cows (o) and HR cows (x)

**Conclusions** Megalac at a higher rate of 60g/kg significantly increased milk yield when fed for the first 16 weeks postpartum without affecting the fat or protein content of the milk. Increased milk yield did not have a negative effect on milk composition or fertility. The HR cows were able to maintain a higher BCS at turnout compared to the LR cows. This was probably due to the higher energy density diet of the HR cows enabling them to balance the deficit in energy requirements of high-protein spring grass.

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

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**Introduction** 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 yields of high starch maize silage in Northern Ireland. However, there is considerable variability in the quality of maize and grass silages produced. In a recent study Keady *et al.* (2002) concluded that the highest yield of fat and protein from dairy cows was obtained from maize silage ensiled at approximately 30% dry matter (DM) when offered as 40% of the forage component of the diets consisting of either medium or high feed value grass silages supplemented with concentrates. Also Keady *et al.* (2002) concluded that replacing 40% of the grass silage component of the diet with maize silage had a concentrate sparing effect, as determined for milk yield, of up to 1.9 kg/cow/d. The objective of the current study was to examine further the effect of maturity of maize silage at harvest on the performance of dairy cattle offered grass silages differing in feed value. The potential concentrate sparing effect of contrasting maize silages was also examined.

**Materials and Methods** Three grass silages, which were harvested from the primary growth of perennial ryegrass swards on 21 May and 14 and 21 June respectively, were offered as the sole forage supplemented with either 7 or 11 kg concentrates/cow/d. Furthermore four maize silages were offered in addition to each of the three grass silages at a ratio of 40:60 maize:grass silage and supplemented with 7 kg concentrates/cow/d. The 18 treatments were offered to 54 lactating dairy cattle in a partially balanced, changeover design study using the Genstat REML (Residual Maximum Likelihood) procedure. The maize silages were produced from crops planted on either 3 or 23 May, either with or without complete cover plastic mulch cover and ensiled between 29 October and 9 November. 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 grass and maize silages are presented in Table 1. The feeding value of the grass silages differed considerably, as determined by DM concentration and predicted D-value. The DM concentrations of the four maize silages differed dramatically. Increasing silage feed value increased ( $P<0.001$ ) the yields of milk and fat plus protein by 3.71 and 0.42 kg/cow/d respectively. The effect of maturity of maize at harvest on animal performance is presented in Table 2. Regardless of maize silage DM concentration, including maize silage increased total DM intake, the yields of milk and fat plus protein and milk protein concentration relative to grass silage offered as the sole forage supplemented with 7 kg concentrate. Increasing concentrate feed level increased ( $P<0.001$ ) total DM intake; the yields of milk and fat plus protein and milk protein concentrations. There was a significant interaction between maize silage DM concentration and grass silage feed value for milk fat concentration. With the high and medium feed value grass silages, increasing maize silage DM concentration decreased fat concentration. However with low feed value grass silage milk fat concentration tended to be higher for the 249 and 429 g/kg DM maize silage relative to the 189 and 362 g/kg DM maize silages. The potential concentrate sparing effect of including maize silage in the diet, as determined for milk yield were 1.7, 1.9, 2.6 and 2.2 kg concentrate/cow/d for maize silage DM of 180, 250, 350 and 400 g/kg respectively.

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

	Grass silage feed value			Maize silage			
	Low	Medium	High	1	2	3	4
Dry matter (g/kg)	218	234	307	189	249	362	429
pH	3.9	3.6	3.9	3.7	3.9	4.2	4.1
Crude protein (g/kg DM)	97	107	148	103	97	87	77
Ammonia (g/kg N)	96	70	65	68	72	70	66
Predicted D-value (g/kg DM)	650	680	730	-	-	-	-

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

	Conc. (kg/d)		Maize silage (MS) DM (g/kg)				Significance			
	7 kg	11kg	189	249	362	429	Sem	Treat.	MS DM linear	GS x MS
Total DMI (kg/d)	17.5 <sup>a</sup>	20.2 <sup>d</sup>	18.3 <sup>b</sup>	18.2 <sup>b</sup>	19.1 <sup>c</sup>	18.7 <sup>bc</sup>	0.18	***	**	NS
Milk (kg/d)	27.9 <sup>a</sup>	31.5 <sup>c</sup>	28.6 <sup>b</sup>	28.6 <sup>b</sup>	29.1 <sup>b</sup>	28.9 <sup>b</sup>	0.24	***	NS	NS
Fat (g/kg)	39.7 <sup>a</sup>	39.9 <sup>a</sup>	41.2 <sup>bc</sup>	41.6 <sup>c</sup>	39.8 <sup>a</sup>	40.2 <sup>ab</sup>	0.45	**	**	*
Protein (g/kg)	32.6 <sup>a</sup>	33.5 <sup>b</sup>	33.3 <sup>b</sup>	33.5 <sup>b</sup>	33.4 <sup>b</sup>	33.6 <sup>b</sup>	0.19	**	NS	NS
Fat + protein (kg/d)	2.01 <sup>a</sup>	2.30 <sup>c</sup>	2.12 <sup>b</sup>	2.14 <sup>b</sup>	2.12 <sup>b</sup>	2.12 <sup>b</sup>	0.021	***	NS	NS

**Conclusions** Regardless of maturity at harvest, maize silage increased food intake and fat plus protein yield when offered with three grass silages differing dramatically in feed value. Feeding maize silage had a concentrate sparing effect of up to 3.2 kg/cow/d, depending on the feed value of the maize and grass silages.

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Keady, T.W.J., Mayne, C.S. and Kilpatrick, D.J. (2002). *Proceedings of the British Society of Animal Science*, p.16.

# The response to concentrate supplementation of dairy cows grazing late summer/autumn grass

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**Introduction** While the milk yield response of spring calving dairy cows to concentrate supplementation during the main summer grazing period has been examined in a number of studies, there is little information available concerning the milk yield response to supplementation in late summer/early autumn. With milk yield at this stage of the lactation having declined considerably, supplementation might appear unnecessary. However, herbage quality and availability are also lower in late season, and as such, responses to concentrate supplementation might be expected. This study was conducted to examine the milk yield response to concentrate supplementation of dairy cows grazing late summer/autumn grass.

**Material and methods** The study involved sixty Holstein-Friesian dairy cows, with animals an average of 178 (s.d.30.0) days calved at the start of the study. Due to the very difficult climatic conditions during the main summer period, animals involved in this study were housed periodically (both by night, and by day and night) from June through to early August, with 6.0 kg concentrate/day being offered during this pre-experimental period. During the seven day period prior to the start of the study, animals were adjusted onto their experimental concentrate levels. The study commenced on the 23 August, and involved an examination of four concentrate feed levels, namely 0.5, 3.0, 6.0 and 9.0 kg concentrate/cow/day. The ingredient composition of the concentrate offered (kg/t air dry basis), was as follows: barley 120, maize meal 210, molassed sugar beet pulp 350, soya bean meal 250, mineral/vitamin mix 25 and molasses 45. These concentrate allowances were offered in-parlour during milking, with the daily concentrate allocation being divided between two equal feeds, at morning and evening milking. Animals remained on these concentrate feed levels for a 50 day period, until 12 October. During this time the cows were grazed in a single group, and given access to fresh pasture daily, following evening milking. Pre- and post-grazing sward heights were measured daily using a rising plate meter, while a pluck sample of pasture offered was taken weekly, and analysed for N and metabolisable energy (ME) concentration, the latter using NIRS. Performance data from this study was analysed using Analysis of Variance.

**Results** Mean pre- and post-grazing sward heights, measured daily during the study, were 12.5 (s.d. 1.82) and 6.2 (s.d. 1.26) cm respectively, while the mean area grazed on a daily basis was 0.4 ha. Grazed herbage had a mean crude protein content of 172 (s.d. 44.6) g/kg DM, and a mean predicted ME concentration of 10.6 (s.d. 0.26) MJ/kg DM. The concentrate offered had a crude protein content of 210 (s.d. 2.8) g/kg DM. The milk production data presented in Table 1 represents mean data for the 50 day period over which the study was conducted. Milk yield increased significantly ( $P < 0.001$ ) with increasing concentrate feed level, however neither milk fat nor milk protein content were significantly affected by treatment ( $P > 0.05$ ). Treatment had no significant effect on either live-weight or condition score, as measured at the end of the study ( $P > 0.05$ ). The mean milk yield response to concentrate supplementation across the 50 day experimental period, based on treatment mean data, was tested and found to be linear. This response is described by the equation:  $y = 0.73x + 14.0$  ( $r^2 = 0.99$ ), where  $y$  = milk yield (kg/day) and  $x$  = concentrate intake (kg fresh/day). Thus the mean milk yield response was 0.73 kg milk/kg fresh concentrate offered. In addition, the data highlights that late summer/autumn grass, when offered as the sole feed to mid lactation dairy cows under relatively tight grazing conditions, has the potential to sustain a milk yield of 14.0 kg/cow/day.

**Table 1** Response of dairy cows grazing late summer/autumn grass, to concentrate supplementation

	Level of supplementation (kg/day)				SEM	Sig
	0.5	3.0	6.0	9.0		
Milk yield (kg/day)	14.3	16.0	18.7	20.3	0.75	***
Milk composition						
Fat (g/kg)	45.2	45.1	44.5	44.5	1.72	NS
Protein (g/kg)	36.3	36.0	36.3	35.8	0.81	NS
Energy (MJ/kg)	32.7	32.8	33.1	33.3	0.43	NS
Final live-weight (kg)	547	539	538	554	16.7	NS
Final condition score	2.34	2.36	2.37	2.39	0.036	NS

**Conclusions** Spring calving dairy cow given access to late summer/autumn grass showed a linear response to concentrate supplementation, with this response being equivalent to 0.73 kg milk/kg fresh concentrate. The economics of this response will depend entirely on the value of milk produced, relative to the cost of the concentrate supplement. In addition, grazed grass at this time of the year, when offered as the sole feed, was found to sustain a milk yield of 14.0 kg/cow/day. However supplementation at this stage of the lactation (day 175 - 225) with high genetic merit Holstein-Friesian cows would appear to provide little opportunity to substantially increase the cows body tissue reserves, with the additional nutrients offered tending to be largely partitioned towards milk production.

# Effect of incremental replacement of cereal grain with sugar beet feed on the lactational performance of high yielding cows offered maize silage based diets

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**Introduction** Dairy cows are increasingly being fed high levels of starch to increase energy intake and promote milk protein content. However, too high levels of degradable starch can cause rumen acidotic effects leading to poor rumen health, laminitis and ultimately reduced efficiency of milk production. Fibrous feeds have known benefits on rumen health by helping to buffer rumen pH, but little is known of the effects of replacing starch with fibre in high maize silage based rations for dairy cows. The objective of this trial was to determine the effects of incremental replacement of cereal grain with sugar beet feed in a total mixed ration on dry matter intake, milk yield, milk composition and yield of milk constituents of lactating dairy cows receiving a high starch diet based on maize silage.

**Materials and methods** Sixteen multiparous Friesian-Holstein cows were used in a 4 x 4 Latin square design study with 4-week periods. The first three weeks of each period were used for adaptation to the newly introduced diet with data for statistical analysis collected during the fourth week of each period.

During the experimental period cows were offered one of four diets (T240:0, T180:60, T120:120, T60:180) all containing maize and grass silage on a 4:1 DM ratio, soyabean meal and rapeseed meal at 120 and 78 g/kg DM respectively and cracked wheat and sugar beet feed in ratios of 240:0, 180:60, 120:120 and 60:180 g/kg DM, respectively. All diets contained 12 g/kg DM of minerals. The concentrate to forage DM ratio was 45:55. Based on the values of the individual ration components the nutritive value of the TMRs are shown in Table 1. The diets were iso-energetic and iso-nitrogenous with only the starch and fibre portions of the dietary treatments changing.

**Table 1.** Nutritive values of the TMRs used in the study based on the analysis of the constituents

	T240:0	T180:60	T120:120	T60:180
ME (MJ/kg DM)	11.9	11.9	11.8	11.8
Crude protein (g/kg DM)	169	167	165	163
Neutral detergent fibre (g/kg DM)	293	305	317	330
Starch (g/kg DM)	290	254	219	183
Sugars (g/kg DM)	51	60	69	78
Oil (AH) (g/kg DM)	32	31	30	30
Ash (g/kg DM)	60	64	67	71
Ratio of NDF:Starch	50:50	55:45	59:41	64:36

**Results** There was a significant ( $P < 0.001$ ) positive linear effect of dietary sugar beet feed on DM intake ( $0.113 \pm 0.0234$  per kg inclusion/100 kg DM) (see Table 2). While DM intake was similar for cows fed T1 and T2 (0 and 60 g/kg DM sugar beet feed) they were 1-2 kg lower ( $P < 0.05$ ) when compared with cows fed T3 and T4 which contained 120 g/kg DM and 180 g/kg DM sugar beet feed, respectively. There were no significant differences between dietary treatment mean values for milk yield or milk composition or yield of milk constituents.

**Table 2.** Dry matter intake, milk yield, milk composition and yield of milk constituents

	T240:0	T180:60	T120:120	T60:180	s.e.d.
DM intake (kg/d)	22.4	22.4	23.7	24.2	0.44
Milk yield (kg/d)	31.7	31.7	31.5	30.8	0.65
Milk fat (g/kg)	41.7	40.9	42.5	41.7	1.37
Milk protein (g/kg)	34.6	34.5	34.9	34.4	0.30
Milk lactose (g/kg)	46.2	46.6	46.1	46.6	0.25
Milk fat (g/d)	1316	1299	1331	1280	50.3
Milk protein (g/d)	1092	1091	1092	1054	21.0
Milk lactose (g/d)	1467	1480	1454	1435	32.3

**Conclusions** In high starch diets based on maize silage, sugar beet feed can be used to replace cracked wheat and form up to 120 g/kg DM of total dietary DM without compromising either milk yield or milk quality. While the current short term study could not provide data on health related benefits of including sugar beet feed in dairy cow diets, it is reasonable to hypothesise that this might occur, based on earlier studies, and that these benefits are likely to be more pronounced in dairy cows receiving high starch based diets.

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

# The effects of *Saccharomyces cerevisiae* on feed intake, milk yield and composition in lactating Holstein cows

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**Introduction** During the recent years, the use of yeast products as feed additives has been increased in animal nutrition (Newbold *et al* 1996). Results from various experiments indicate that these microorganisms are able to alter ruminal fermentation patterns and have positive effects on the performance of ruminants (Wolht, *et al.* 1998). Therefore, the aim of this experiment was to investigate the effects of Biosaf, a live yeast strain of *saccharomyces cerevisiae* (Sc 47), on feed intake, milk yield and composition in lactating Holstein cows.

**Materials and Methods** Twenty four Holstein dairy cows (at  $20 \pm 2$  weeks of lactation) were used in a 2x2 Latin square changeover design with 2 four-week periods. The animals were assigned into two groups based on milk yield, calving date and parity and received either a basal (Control) or yeast supplement (Biosaf) diet after a 10 days adaptation period in individual stalls for the duration of the study. Basal diet contained of *ad libitum* access to Lucerne hay and barley straw (2.7: 0.9 kg ratio respectively), corn silage 3.5 kg, and concentrate 9.5 kg on dry matter basis. Concentrate was fed in three equal portions immediately after milking and silage was offered at 15.00h. The concentrate contained (g/kg) ground barley 415, wheat bran 260, cottonseed meal 220, dried beet pulp 85, and mineral/vitamin supplement 20. The only variable for treatment group was the addition of 10g supplemental Biosaf<sup>®</sup> ( $5 \times 10^9$  CFU of *Saccharomyces cerevisiae* Sc 47, provided from Lesaffre) which were top-dressed on the concentrate of morning feeding. Cows were milked three times per day at 6.00, 14.00, and 22.00 h and milk volume was recorded at each milking time. Milk samples were collected from morning milking for each cows once a week for analysis of fat and protein. Chemical composition of diet ingredients was determined and feed intake was also measured for each cow daily. Data were analysed by analysis of variance using Minitab.

**Table 1** Chemical composition of feed ingredients

Ingredients	DM g/kg	g/kg dry matter		
		CP	NDF	ADF
Lucerne hay	920	155	435	345
Barley straw	935	33	755	475
Corn silage	334	72	652	388
Barley grain	915	920	235	135
Wheat bran	920	155	455	135
Cottonseed meal	935	325	405	305
Sugar beet pulp	940	75	395	195

**Table 2** Effects of yeast supplementation on feed intake, milk yield, and composition in the dairy cows.

	Biosaf	Control	Sem	Sig
DM intake (kg/d)	17.7	17.8	0.62	NS
Milk yield (kg/d)	19	18.7	0.57	NS
4% FCM (kg/d)	17.8	17.1	0.54	*
<b>Milk composition (g/kg)</b>				
Fat	3.6	3.4	0.10	NS
Protein	3.8	3.7	0.09	NS
<b>Milk component yield (g/d)</b>				
Fat	679	639	13.3	NS
Protein	731	682	24.2	NS

\*P<0.05 FCM = fat-corrected milk

**Results** Chemical composition of diet ingredients are shown in Table 1. Yeast supplementation had no effect on the dry matter intake although milk production tended to be higher in cows receiving Biosaf compared to the control diet. During the experimental periods, the mean values of milk yield in treatment group were from 0.2 to 0.8 (litre/day/head) higher than that of control group. The differences between the amounts of milk yield in cows fed Biosaf compared to the control group were statistically significant ( $p < 0.05$ ) when milk yield expressed on 4% fat-corrected milk (FCM). The yields of milk fat and protein were also higher in treatment group. The mean values of these parameters in Biosaf diet were 679 and 731 g/d, compared to the control diet, which were 639 and 682 g/d respectively (Table 2). However, the differences were not statistically significant.

**Conclusions** The results of this study indicate that the addition of *Saccharomyces cerevisiae*, Sc 47 may result in a higher amount of milk yield and yield of milk fat and protein without any effect on feed intake in lactating Holstein cows. This could be attributed to the positive effects of live yeast culture on rumen microbial activities which affect feed digestibility in the rumen and in turn may results the efficiency of feed to be improved.

**Acknowledgement** The financial support of Makian Daroo Co. and the help of the staff of Aminabad research institute of veterinary faculty is kindly acknowledged..

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## Effect of season and parity on the milk production of Iranian Holstein cows

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**Instruction** The variation in milk production is a regular phenomenon in all milking animals, broadly the factors which are responsible for such variations can be divided into 1) Physiological, which will be governed by the genetically make up and 2) Environmental, such as age, number of previous lactations, pregnancy, season of calving, calving interval and nutrition status. The season of calving has got a marked effect on the total production. The objective of this study was to investigate the effect of season of calving and parity of Iranian Holstein dairy cows on the milk production.

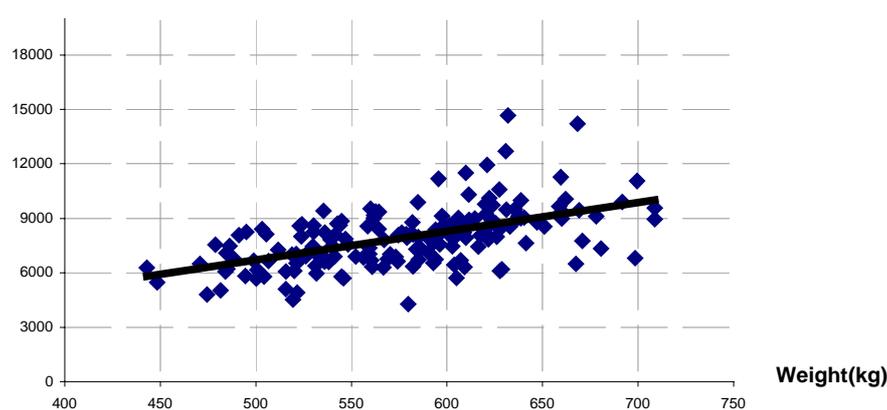
**Materials and methods** This experiment was carried out in the dairy farm of Ferdowsi University of Mashhad, which is situated in the northeast of Iran with his geographical characteristics (59.39-36.20), between years (1995-2001). Milking cows had been divided between two groups over (>30 Kg) and under (<30 Kg). Milk production from one hundred cows was individually measured in the three times day during this experiment. The milking cows diets were calculated to supply requirements according to NRC 1989 and were offered as TMR in three times a day. Milk production of each cow was adjusted for 305 days (3X) according the factors submitted by DHI. All cows that had problems during lactation such as chronic mastitis, laminitis were omitted from data. The live weight of cows was measured on the last days of each month during experiment period. The data were analyzed by analyses of variance of a completely randomized design with unequal replications and differences between means were tested with Duncan test.

**Results** As can be seen from table 1, there was a significant effect between milk productions in the different seasons. The highest level of milk production was in the winter and the lowest was in the summer. The reason is probably due to weather temperature during years (Bernabucci et al., 2002). Figure 1 also shows the relationships between body weight and milk production. Larger cows have more udder secretory tissue and larger digestive systems, therefore, produce more milk (Collard, B. L., 1999). There was a significant difference between milk productions in different lactations (Table 1) due to less udder secretory tissue and lower digestive capacity.

**Table 1** Effect of season, parity on the milk production

Season	1	2	3	4	SEM
Milk(305 day)	7643 <sup>b</sup>	7538 <sup>b</sup>	8149 <sup>a,b</sup>	8495 <sup>a</sup>	214.44
Milk (45 day)	1201 <sup>b</sup>	1227 <sup>b</sup>	1215 <sup>b</sup>	1371 <sup>a</sup>	32.96
Milk (90 day)	2553 <sup>b</sup>	2566 <sup>b</sup>	2582 <sup>b</sup>	2893 <sup>a</sup>	56.59
Milk(120 day)	3454 <sup>b</sup>	3385 <sup>b</sup>	3473 <sup>b</sup>	3866 <sup>a</sup>	72.15
Parity	1	2	>3		SEM
Milk (305 day)	7045 <sup>c</sup>	8029 <sup>b</sup>	8740 <sup>a</sup>		185.71
Milk (45 day)	994 <sup>c</sup>	1296 <sup>b</sup>	1444 <sup>a</sup>		28.54
Milk (90 day)	2160 <sup>c</sup>	2719 <sup>b</sup>	3013 <sup>a</sup>		49.01
Milk (120 day)	2941 <sup>c</sup>	3634 <sup>b</sup>	3990 <sup>a</sup>		62.48

production(Kg)



**Figure 1** Relationship between body weight and milk production

**Conclusion** It has been shown that milk production is less during summer. Parity and larger cows has a positive effect on milk production.

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## A feasibility study on the automatic recording of condition score in dairy cows

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**Introduction** The use of body tissue by dairy cows to support lactation is increasing, since selection has led to cows that can eat only around half of their incremental feed requirements per unit increase in genetic merit for milk production (Veerkamp et al., 1995). Continuing with this selection policy is likely to result in increasing use of body tissue to fuel milk production and to lead to thinner cows with associated health and fertility problems. This has created increasing interest in body condition scoring (CS) in dairy cows as both an important management tool and also for use in selection indices. The ability to automatically record CS would increase the use of this measure in farm management and enable large volumes of data to be collected for use in national evaluations.

**Materials and Methods** Structured red laser light (wavelength 650nm) was used to create light lines across the tail-head area of the cow. A digital camera was mounted on the same assembly and positioned to be at 45° to the horizontal plane of the cows back. The complete assembly was mounted on a sliding rail that allowed optimum positioning of the light array with respect to the cow, and the overall geometry of the projected light and the camera was therefore maintained. Prior to the start of the experiment, the exact areas of the cow that contained potential information were not known and so previous experience and published results was used to focus on the area of the tail head. After image enhancement, points on the laser stripes were clicked off using a mouse. For each stripe, two quadratic curves were fitted through the data: one through the points in the tail head region, and one through the points on the right buttock. In effect, this assumed that the shape of the cow, from left to right, could be approximated by three quadratic humps: one for each buttock and one for the tail head.

**Results** Thirty six cow images were of suitable quality for the laser stripes to be extracted. 224 stripes were extracted from these, an average of 6 stripes per cow. Two measures of shape were tested for their correlation with condition score. The curvatures of the tail head and right Pin bone were selected because they were thought to be measures of the “boniness” of the animal (Figure 1). The correlation between tail head curvature and condition score was 0.55 and between Pin bone and condition score was 0.59 (Figure 2).

Figure 1. Typical fit of model to cow shape (tail head and right buttock) across the pin bone: broken line, laser stripe data; solid line, fitted quadratics

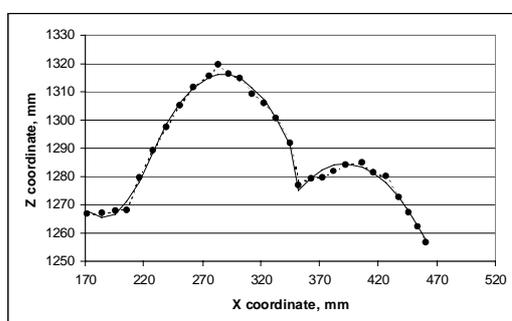
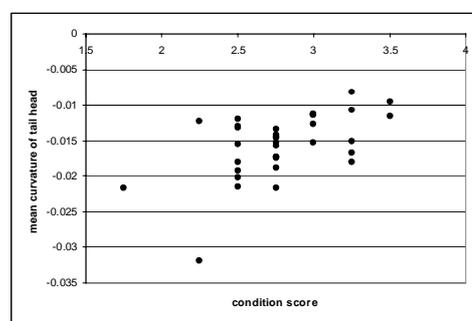


Figure 2. Mean tail-head curvature and CS



**Conclusions** It is feasible to extract shape information on cows from digital images. Those shapes are correlated to CS. Given the difficulty in emulating visual cues from digital images, a more realistic objective to pursue would be to find parameters of a shape (or combination of shapes) that is related to body fatness itself and then to relate that shape parameter to CS. In this project, the work set out to predict CS which is itself a predictor of body fat content. The system of determining CS (Lowman et al., 1976) was developed for visual appraisal of beef cows and exploits the human eye's ability to assimilate a large number of interacting and subtle cues. Expecting software to be able to emulate that process might be unrealistic in the short term but defining a new system especially for digital image capture may make body energy assessment from digital images more feasible in future. This might involve digitally captured shape data from more than one site in conjunction with historical data for animals already in the herd, or standard curves for new animals, combined with information from relatives. Reducing the impact of the shape data alone may allow more opportunities for continued research into extracting shape data whilst allowing for the best use to be made of all available data. An automated body fat content assessment system would be an invaluable addition to an integrated management system and would allow more timely management intervention when cows lose body fat.

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## The effect of intravenous infusion of glucose on ethanol stability of milk in Holstein dairy cows

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**Introduction** The alcohol test is used as the initial classification of milk in dairy farms. It's used as a measure of the natural pH of milk, which is a critical factor for stabilizing casein micelles in milk serum phase during heating (Barros et al., 2000). In practical conditions the test could be also positive immediately after milking, and this type of milk is rejected by dairy processing industry. An experiment was conducted to evaluate the effects of negative energy balance and low level of blood glucose on incidence of alcohol-positive milk in Holstein high milking cows (Sobhani et al., 2002).

**Materials and methods** Four Holstein-Friesian dairy cows in mean lactation stage of 98 days (s.d. 32) and average milk yield of 35 kg/d (s.d. 2.8) and mean ethanol stability of 58% (s.d. 3) were used. Four central venous catheters were inserted in jugular vein of these cows and were housed in individual tie stalls. The cows were adapted to the new conditions during the five-days pretreatment period. The cows were infused in two consecutive two-day periods with 100 and 200 g/d glucose solution through jugular vein respectively. Solutions were infused at the rate of 2 ml.min<sup>-1</sup> and the experiment was ended after a two-days post-treatment period. At the end of each period blood and milk samples were collected. Milk samples were analyzed for fat, protein, lactose, total solid, casein and pH. Blood plasma samples were analyzed for determination of glucose. Ethanol stability and dry matter intake were measured daily for individual cows. The experimental design used four cows that were randomized in an incomplete Latin square design.

**Results** Mean milk yield and compositions and blood glucose samples are shown in Table 1. No significant difference was observed between infusion periods for fat, protein, lactose and casein content of the milk samples, although total solid content of the milk samples and blood glucose level differed significantly ( $P \leq 0.05$ ) with increasing the level of infused glucose. The ethanol stability of milk samples and dry matter intake decreased by increasing level glucose. These changes are shown in figure 1.

**Table 1** Effects of glucose infusion on some of variables

	Pre. <sup>1</sup>	100 g/d	200 g/d	Post. <sup>2</sup>	s.e.m.
<b>Blood glucose</b>	49	60	66	51	3.00
<b>Ethanol Stability</b>	58	56	54	64	4.00
<b>Milk, kg.d<sup>-1</sup></b>	35.50	35.25	30.75	28.50	4.00
<b>Total Solid %</b>	11.26	11.43	12.03	10.86	0.25
<b>DMI kg.d<sup>-1</sup></b>	20.88	19.83	18.79	20.18	1.00

<sup>1</sup>preinfusion period

<sup>2</sup>postinfusion period

**Conclusion** The lack of improvement in ethanol stability which was the main object of this study, can be attributed to intake reduction as a result of increasing blood glucose, and stress of treatment application in a short period and unfavorable conditions. Therefore, it seems that in a long period and favorable conditions can positively affect ethanol stability in high milking cows.

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# Environmental constraints in habitat use by free-ranging beef-cattle in the Natural Park of Gorbeia (Basque Country)

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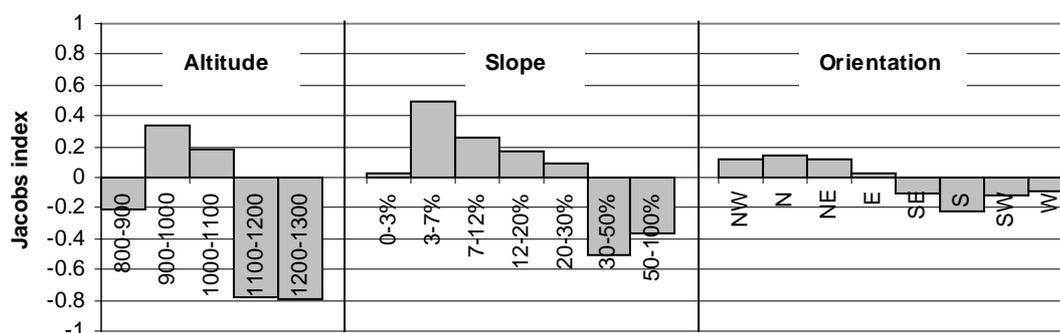
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**Introduction** Mountain pastures utilisation in the Basque Country by means of a free-range mixed-grazing system has suffered substantial changes during the last decades. Beef cattle production has increased partially favoured by CAP (Common Agricultural Policy) measures, mainly milk-quotas and the extensification premium. The use of mountain areas is constrained by both biotic and abiotic factors in cattle (Senft et al, 1985; Bailey, 1995) which are necessary to be identified in order to improve habitat management of these mountain zones, usually declared as protected areas. The objective of the current work was to study the influence of physical factors on the behaviour of beef-cattle herds managed in a transhumant free-range system.

**Materials and methods** The study was carried out in two *management units* (Steward & Eno, 1998) of the Natural Park of Gorbeia characterised by abrupt orography. Vegetation ranged from tree-covered areas to open pastures, mainly composed by *Agrostis capillaris* and *Festuca rubra*, with different degrees of shrubby cover. Two beef-cattle herds grazing these places were monitored during the grazing seasons (from May to November) of 1997 and 1998. Daily habitat location of each herd was monitored in 1-hour intervals by scan-sampling using orthophotos (1:10.000). To establish relationships between beef-cattle's behaviour and environmental constraints, each point was related to topographic conditions (altitude, slope and orientation) by means of GIS (Geographic Information Systems), and the *Jacobs selection index* (Jacobs, 1974) was applied.

**Results** Throughout the two years of study, a total of 28 controls and 365 location points were considered. The most important activity during daylight was grazing, followed by resting and walking (Mandaluniz and Oregui, 2000). According to the *Jacobs selection index*, herds selected positively lower altitude areas, especially those under 1100 masl (Figure 1) due to a combination of factors such as vegetation, animal constraints, presence of other species, etc (Bailey, 1995; Senft et al, 1985). However, the negative selection of 800-900 masl areas was related to the tree-cover area, which used cattle for resting. They also selected positively flat areas or smooth slopes (less than 30%) which shows that slope is an important constraint for these herbivorous (Senft et al, 1985). The positive selection of northward-orientated patches (N, NE and NW) and negative of southward-orientated ones (S, SE and SW) could be related to differences in vegetation characteristics (data not published).



**Figure** Error! Unknown switch argument.. *Jacobs selection index* values for the altitude, slope and orientations of the studied areas.

**Conclusions.** Environmental factors such as altitude, slope and orientation play a significant role in beef-cattle activity. The influence of these factors could be related to pasture conditions which affect grazing activity, but also to physical limitations to animal's mobility (Senft et al, 1985). These influence on animal activity should be affected, also, by the environmental factors of the studied area, so it should be necessary to increase the knowledge of them in different situations and achieve a better understanding of plant communities evolution.

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## A decision support system to evaluate the pollution potential of diets for dairy cows

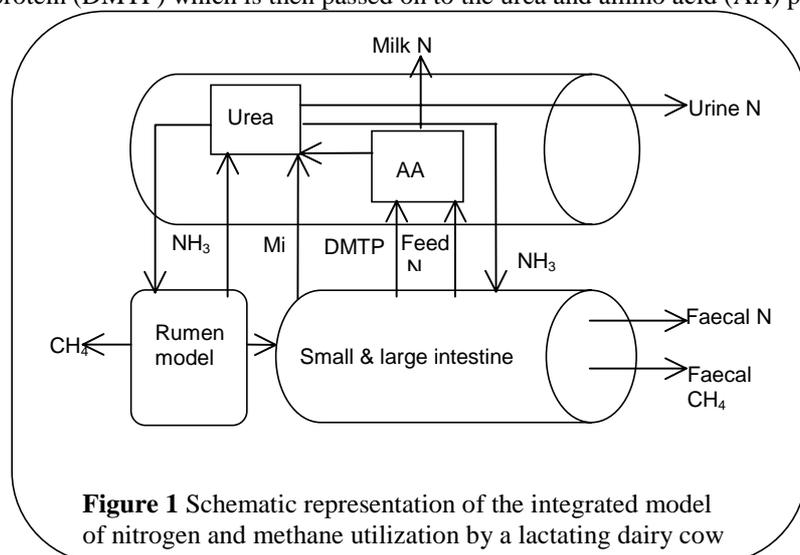
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**Introduction** Agriculture in general, and dairy production in particular, has been identified as one of the major sources of greenhouse gas emissions and other environmental pollutants such as nitrogen (N) (as ammonia, N and Nitrous oxides, and N leaching). Availability of cheap sources of protein has led to increased consumption of protein supplements. However, the protein is often utilised inefficiently and excess nitrogen is excreted particularly in urine, which has much more potential to pollute the environment. One of the obvious ways of reducing pollution is by evaluating the pollution potential of diets and formulating more balanced rations. A few technical mathematical models have been published but rarely do they consider more than one pollutant at a time. The objective of the present study was to develop a decision support system (DSS) by first integrating mechanistic models of N (Kebreab et al., 2002) and methane (Mills et al., 2001) with a rumen model (Dijkstra, 1994) and then develop a graphical user interface (GUI) for ease of use and analysis of outputs.

**Model Development** Originally the technical models were developed using ACSL, the advanced continuous simulation language. The individual models were re-coded in Visual Fortran to harmonize the models. In order to bring the models to the same level of complexity, reclassification of some of the pools were necessary. For example, in the N model of Kebreab et al. (2002), the microbial N was estimated by a Michaelis-Menten equation which was dependent on urea N. In the DSS, the rumen model estimates the production of microbial N (including non-protein N (Mi) and digestible true protein (DMTP) which is then passed on to the urea and amino acid (AA) pools in the N module.



**Figure 1** Schematic representation of the integrated model of nitrogen and methane utilization by a lactating dairy cow

The model contains 40 pools; 19 of which are in the rumen and the rest in the lower tract, blood and PDV. A simplified representation of the model is given in Figure 1. The GUI was developed with Visual Basic 6 and it allows the user to input dietary details required by the model. The model is run until steady-state is achieved. Various numerical integration methods are available with a fourth-order Runge-Kutta being the default method. After the model is run, it provides a summary of results showing N balance and methane outputs. The DSS also provides the user with the option to analyse data using plotting software.

**Results** The DSS model was able to predict methane and N outputs with the same reliability as the individual models, but with the added advantage of comparing the relative reductions in N and methane emissions. For example, a cow consuming 18 kg of a typical diet containing 16% CP and 12 MJ/kg DM ME was predicted to excrete 161 g N in urine, 144 g N in faeces and 398 g in methane emissions. Increases in N intake without matching energy consumption resulted in an exponential increase in urine N and significant increases in methane gas emissions. This is mainly as a result of less N capture by microbes in the rumen and hindgut which led to increased levels of urea-N in blood which were excreted in urine. The type of energy used in the diet had a significant effect on both N and methane pollution. Simulations of feeding cows with slowly degradable starch (e.g. maize) showed significantly lower methane emissions and N excretion compared to cows fed high degradable starch sources.

**Conclusions** The DSS model can be used to predict the potential pollution from a diet and also to optimize a diet for improved utilization by the animal and thereby reduce N and methane emissions. The model can also be used to estimate emissions at farm or at national levels and evaluate options for mitigating nutrient pollution from dairy cows.

**Acknowledgements** The authors appreciate financial support provided by DEFRA Agri-Environment (WA0320).

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# The Mitscherlich equation: an alternative to linear models of methane emissions from cattle

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**Introduction** Previous attempts to apply statistical models, which correlate nutrient intake with methane production, have been of limited value where predictions are obtained for nutrient intakes and diet types outside those used in model construction. Dynamic mechanistic models have proved more suitable for extrapolation, but they remain computationally expensive and are not applied easily in practical situations. The first objective of this research focussed on employing conventional techniques to generate statistical models of methane production appropriate to UK dairy systems. The second objective was to evaluate these models and a model published previously using both UK and North American datasets. Thirdly, non-linear models were considered as alternatives to the conventional linear regressions. The UK calorimetry data used to construct the linear models were also used to develop the three non-linear alternatives that were all of modified Mitscherlich (monomolecular) form.

**Model Development** Data from 11 trials ( $n = 159$ ) conducted at the Centre for Dairy Research (CEDAR) UK, were used to develop the models. The backward elimination procedure for multiple regression in SAS was used to produce the linear models and the criteria for selecting the best-fit model were as described by Oldick et al. (1999). The main effects were analysed using Proc Mixed procedure of SAS (2000). The best fit model, Linear 1, was as follows:

(Linear 1);  $CH_4$  (MJ/d) = 5.93(SE 1.60) + 0.92(SE 0.08) x DMI (kg/d) ( $r^2=0.60$ ; RMSE = 1.82)

For the non-linear modelling, the Mitscherlich equation was reparameterised with as follows:

$$y = a - (a + b)e^{-cx}$$

where  $a$  and  $-b$  are the maximum and minimum values of  $y$  respectively, and  $c$  is a shape parameter determining the change of  $y$  with increasing  $x$ . The data were gathered from multiple trials so a meta-analytic approach was adopted. Therefore, non linear mixed procedure (PROC NL MIXED in SAS, Littell et al. 1996) which took into account the fixed and random effects of trials (and their interaction) was used to fit parameter values to the CEDAR data (see results section). Mitscherlich 1 used DMI as the independent variable while Mitscherlich 2 and 3 used MEI. For all three models, parameter  $b$  was fixed at zero representing cessation of methanogenesis at nil intake. Parameter  $a$  represents the maximum potential methane production and was fitted for each model against the CEDAR dataset using the NL MIXED procedure giving values of 56.27, 45.98 and 45.98 for Mitscherlich 1, 2 and 3 respectively. For Mitscherlich 1 and 2,  $c$  was fitted to these data using the same procedure giving values of 0.028 and 0.003 respectively. Previous research has established a tendency for fibrous diets (high ADF) to increase methane, while the reverse is true for diets high in starch. Therefore for Mitscherlich 3,  $c$  represents the ratio of dietary starch to ADF as follows:

$$c = -0.0011 \times \left[ \frac{\text{Starch}}{\text{ADF}} \right] + 0.0045 \quad (r^2 = 0.97)$$

**Results** Table 1 shows that for the American data all models produced significant overestimates of methane production when evaluated against the American data. The models split into two groups according to the magnitude of this over prediction. Linear 1 and Mitscherlich 1 over predicted emissions for these data by between 31% and 39%. Whereas, Mitscherlich 2, 3 and Moe and Tyrrell (1979) gave improved estimates of between 17% and 26% above the observed mean. The lowest error of prediction was demonstrated by Moe and Tyrrell (1979) and Mitscherlich 3. For the UK data, all models except Mitscherlich 3 under predicted methane production. Also, the degree of deviation from the observed mean was reduced with the greatest under prediction being only 12% (Moe and Tyrrell, 1979).

**Table 1** Summary of observed vs. predicted methane production for various models

Model	Linear 1	Moe & Tyrrell	Mitscherlich 1	Mitscherlich 2	Mitscherlich 3
Dataset	Predicted Mean methane (MJ/d)				
American (obs = 12.4)	17.34	15.61	16.28	15.26	14.57
UK (obs = 20.8)	18.84	18.49	18.14	19.40	22.15
	root MSPE (%) <sup>1</sup>				
American	43.7	34.0	40.0	36.0	34.1
UK	19.3	17.5	20.2	17.2	16.3

<sup>1</sup>Square root of Mean Square Prediction Error expressed as a percentage of the observed mean.

**Conclusions** The non-linear models, in particular Mitscherlich 3, were most successful at predicting methane production across a range of production systems. They are less prone to misapplication than their linear alternatives while providing an improved level of prediction across a range of diets. These advantages are without any additional requirements for details of diet composition.

**Acknowledgements** The authors appreciate financial support provided by DEFRA Agri-Environment (WA0320).

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# Estimation of the effects of some environmental factors and genetic parameters of linear type traits in Holstein cows of Iran

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**Introduction** The primary emphasis in dairy cattle selection is for yield traits because highest producing cows usually are more profitable. Selection on yield traits alone could decrease merit for other traits. Selection on type traits associated with increased herd life may be beneficial to decrease involuntary culling and increase profitability. One of the primary reasons for collecting and utilizing information on type is to aid breeders in selecting profitable, functional cows. So that early culling for causes unrelated to yield (involuntary culling) can be avoided [Misztal et al, 1992]. The objectives of the present study were to evaluate the effects of some environmental factors on and to estimate genetic parameters of some type traits.

**Material and methods** Records on 550 Holstein dairy cattle from two herds, located in Tehran province of Iran, were available. Type traits were described with numerical scores on a 50-point scale, beginning at 50. Data were analyzed by method of least squares fitting constants. The model for the analysis included herd and month of classification as fixed effects and sires within herds and residual error as random effects. Linear and quadratic effects of age (in month) and stage of lactation at classification (in days) were also included as covariables. (Co) variance components were estimated by method 3 of Henderson. Estimates of genetic parameters were based on paternal half sibs' correlations. All analyses were carried out using LSMLMW program [Harvey, 1987].

**Results** Means for linear type traits ranged from 67.8 (Fore udder attachment) to 78.9 (Body depth) with standard deviations between 1.73 (Pastern) and 9.03 points (Fore udder attachment). The 78.9 average for body depth indicates that in view of the classifier cows were nearly deep in the middle of the body and chest. The 67.8 average for fore udder attachment implies that cows had weak fore udder attachment. Herd, month, age and stage of lactation had significant effects on most of the traits ( $p < 0.05$ ). Phenotypic correlations between type traits were generally low (average 0.15), and ranged from -0.23 (between udder depth and rear udder width) to 0.89 (between strength and body capacity). Genetic correlations were generally higher than phenotypic correlations in absolute value, and were in the range of 0.75 (between fore udder attachment and udder support) to 0.98 (between dairy character and final score). Most of the traits had high genetic correlations with final score. This result suggests that selection on final score alone is an efficient means of achieving a wide range of goals in improving type traits. The heritabilities of the traits under study were as follows (Table 1).

**Table 1** The means and the heritabilities of the type traits under study and their corresponding standard errors

Trait	Mean (SE)	Heritability (SE)	Trait	Mean (SE)	Heritability (SE)
Stature	77.0 (4.34)	0.31 (0.22)	Rear udder height	75.0 (4.74)	0.12 (0.15)
Strength	76.9 (4.83)	0.22 (0.19)	Rear udder width	77.2 (5.33)	0.11 (0.15)
Body depth	78.9 (5.70)	0.26 (0.21)	Udder support	73.3 (6.83)	0.11 (0.16)
Angularity	75.4 (3.62)	0.30 (0.22)	Udder depth	73.0 (6.23)	0.20 (0.18)
Front end	75.1 (3.81)	0.08 (0.15)	Fore udder length	72.3 (6.13)	0.00 (0.04)
Shoulders	72.7 (4.06)	0.04 (0.13)	Udder balance	72.3 (3.39)	0.27 (0.21)
Back	75.6 (3.51)	0.00 (0.04)	Teat placement (rear view)	67.0 (5.99)	0.26 (0.16)
Rump angle	77.1 (3.97)	0.26 (0.20)	Teat placement (side view)	75.8 (5.50)	0.15 (0.17)
Rump length	76.9 (3.08)	0.16 (0.16)	Teat size	75.3 (3.68)	0.12 (0.16)
Rump width	75.0 (4.29)	0.22 (0.18)	General appearance	75.9 (2.23)	0.20 (0.18)
Rear legs (side view)	74.0 (2.58)	0.04 (0.12)	Dairy character	76.5 (2.66)	0.25 (0.20)
Rear legs (rear view)	74.6 (3.78)	0.10 (0.15)	Body capacity	77.0 (4.07)	0.26 (0.21)
Pasterns	75.2 (1.73)	0.09 (0.16)	Mammary system	72.8 (2.78)	0.18 (0.18)
Fore udder attachment	67.8 (9.03)	0.13 (0.17)	Final score	75.4 (2.10)	0.24 (0.20)

**Conclusion** A continuing effort is needed to encourage appraisers to increase the range of appraisal scores for a few traits so that standard deviation for each trait will be near 6 or 7 points. Genetic evaluations of bulls for type traits need to be based on models that take herd, month, age and stage of lactation at classification into account. Moderate genetic improvement is possible for many type traits if selection is directed to a specific trait. Back, fore udder length and rear legs (side view) are exceptions.

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## Possibility of reducing milk recording and sampling in Holstein dairy cattle of Iran

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**Introduction** The Iranian Animal Breeding Center (IABC) is currently milk recording and sampling three times per test-day (8 hours interval) approximately once every month. Milk samples are analyzed for fat and protein contents. There are some difficulties for having precise milk samples in some farms especially for three times sampling per day, and the costs of three times recording and sampling is also a problem. Estimation of lactation yield for milk, fat and protein is done based on test-day records and samples and the accuracy of these estimates depend on the precision of test day records especially milk sampling. The results of milk content are not satisfactory for farmer and specialist in the current milk recording system. Some researches have been done to reduce milk recording and sampling per test day (1, 2). In addition potential benefits of reducing milk recording and sampling are that more herds can be supervised and recorded per month. The objective of this research was to consider the possibility of reducing milk recording and sampling in herds with three times milking per test-day in industrial Holstein farms.

**Materials and methods** 45800 test day records were collected from 12 herds during 1999 to 2001 by the IABC. Data set included records for milk, fat and protein yields, fat and protein percentage. Different regression models were applied to estimate daily milk (6 models) fat (12 models) and protein (12 models) on different combinations of milking and sampling times records. Heritability and genetic parameters were estimated using univariate model ( $y = Xb + Za + e$  in this model  $y$  is milk yield, fat yield or fat %,  $b$  is vector of fix effects that include herd-year-season and age of calving,  $a$  is vector of additive genetic also  $X$  and  $Z$  are design matrix and  $e$  is residual effect) repeated records model ( $y = Xb + Za + Wp + e$  in this model  $p$  is vector of permanent environmental effect) and multivariate animal model (in this model milk and fat yield and fat% were analysed) with DFREML method based on estimated 305 days records.

**Results:** Results of this research shows that daily milk, fat and protein yields could be estimated based on night and noon milking times which are in Table 1. Estimated Heritability for adjusted 305 day milk and fat yield and fat percentage records in first lactation were 0.3075, 0.2037 and 0.3378 and for estimated records (based on models in table 1) were 0.3012, 0.1934 and 0.3667 respectively in univariate models. This results for repeated model including first to 3<sup>rd</sup> lactation records were 0.1801, 0.1813 and 0.3098 for adjusted and 0.1802, 0.1705, 0.2908 for estimated records. Repeatability of these traits were 0.48, 0.464, 0.514 and 0.488, 0.45, 0.51 respectively. Heritability of milk and fat yield and fat percent were 0.2448, 0.1764, 0.2862 and 0.2426, 0.1752, 0.3615 respectively for adjusted and estimated records in multivariate. Rank correlation between breeding value actual and estimated records for milk and fat yield and fat percent were 1, 0.985 and 0.973 respectively.

Table 1- Regression model for estimated milk, fat and protein yield

Trait	Regression equation	R <sup>2</sup> #	MSE*
Milk, Kg	Daily milk = 1.784 + 1.44* night milk + 1.419*noon milk	0.928	4.097
Fat, Kg	Daily fat = -0.314 + 0.146*noon fat% + 0.03387*noon milk + 0.04714*night milk	0.744	0.0188
Protein, Kg	Daily prot. = -0.587 + 0.203*noon prot.% + 0.0448*noon milk + 0.04588*night milk	0.86	0.0100

#Coefficient of determination

\*Mean square of error

**Conclusion** The results of this research show that milk recording and sampling could be reduced to two and one time per day without significant decrease in accuracy of estimated (co) variance components and breeding values.

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## An estimate of heritability of clinical tail biting on a commercial pig breeding farm

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**Introduction** Recent research suggests that the genetic makeup of a pig may contribute to the expression of tail biting (Breuer *et al.*, 2002). To date there has been little investigation into the genetics of tail biting. A significant population of tail biters was found at a commercial pig breeding farm at which an experiment on the genetic basis of the expression of harmful social behaviour was being performed. As pedigree data for each pig on the farm were available, the opportunity was taken to investigate the heritability of tail biting by recording the perpetrators of clinical tail biting.

**Material and Methods** Nine thousand and eighteen pigs (3177 Large White and 5841 Landrace pigs) were observed on a commercial pig-breeding farm from 31/7/2001 to 7/4/2002 to investigate the incidence of clinical tail biting. The behaviour of the pigs in all the flat deck accommodation and grower pens was observed 3 to 5 times a week. When clinical tail biting was observed in a pen, the experimenter stood quietly in front of the pen and observed the behaviour of the pigs for a period of 10 minutes, watching for chewing and chasing of tail behaviour. Any pig that was observed chewing and chasing the tails of penmates for the majority of this 10-minute period was identified as the tail biter and its identity, breed, gender and relative size compared to its penmates were recorded. A chi-squared goodness of fit test with 1 degree of freedom was used to determine if an equal proportion of boars and gilts and an equal proportion of Landrace and Large White pigs were identified as clinical tail biters. The heritability of tail biting, analysed as a 0-1 trait, was estimated using an animal model with DFREML methodology (Meyer, 1988) incorporating pedigree data from all pigs on the farm during the period of data collection. A model was fitted within each breed, which included effects for animal, sex and month of observation.

**Results and Discussion** The overall incidence of tail biting individuals for Large White pigs was 2.8% and for Landrace was 3.5%. Gender did not have a significant effect on the proportion of clinical tail biters observed with a similar number of boars and gilts identified as clinical tail biters (151 vs 144,  $\chi^2_1=0.08$ ,  $P>0.05$ ). Although not significant, more Landrace than Large White pigs tended to be identified as clinical tail biters (205 vs 90,  $\chi^2_1=2.98$ ,  $P<0.10$ ). The heritability of tail biting as a 0-1 trait for Large White did not differ from zero even when the analysis was confined to the 7-month period in which the majority of biters were observed. It was therefore not possible to transform the 0-1 estimate of heritability for Large White to an underlying continuous distribution. The heritability of tail biting as a 0-1 trait for Landrace was estimated to be 0.05+/-0.02. When the Landrace estimate was transformed to an underlying continuous distribution (Dempster and Lerner, 1950) it gave an estimate of heritability of 0.27. This estimate of heritability is moderate compared to other estimates obtained for behavioural traits including maternal aggressiveness towards piglets (0.4 to 0.9, Knap and Merks, 1987) and the occurrence of sitting behaviour in pigs (0.41+/-0.14, McGlone *et al.*, 1991).

**Conclusion** The results suggest that breed may have some influence on the expression of tail biting, with Landrace tending to tail bite more often than Large White under the same farm conditions. Our data demonstrate that clinical tail biting is heritable in Landrace but not in Large White pigs. The heritability of liability to clinical tail biting in Landrace pigs is significant, although lower in magnitude to that found for other behavioural traits in pigs.

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

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# Multivariate REML estimates of genetic parameters of monthly test day milk production traits in first parity Iranian Holstein cows with the use of a repeatability test day model

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**Introduction** In practical dairy cattle breeding programmes, usually a small number of animals (selected from a large population) have a major influence on the genetic gain of the concerned population over a period of time (Hofer, 1998). Candidate animals are usually selected based on their breeding values that are predicted by using animal models. In order to predict breeding values, genetic parameters (calculated from variance and covariance components) of the traits under consideration should be estimated to be used in genetic evaluation systems either based on lactation or test day models. The use of test day models has increasingly become of interest in genetic evaluation of dairy cattle due to the fact that they can take more accurate account of the effects of environmental factors influencing test day milk yield over the course of lactation. The main objective of this study was to use a repeatability test day animal model to estimate genetic parameters of monthly test day milk production traits in first parity Iranian Holsteins.

**Material and Methods** Data comprised 53,673 monthly test day milk records from 6,101 Iranian Holstein heifers calved between 1983 and 1995 and distributed in 174 herds from different climatic regions of Iran. A multivariate repeatability test day model was used to estimate genetic parameters of monthly test day milk production traits over the lactation period. In the model, fixed effect of contemporary groups of Herd-Year-Season of production-Month of lactation (HYSopMol)<sub>it</sub> including individual main effects and all two, three and four-way interactions, linear and quadratic covariates of age of cow at test day (A<sub>ijkt</sub>), random effects of additive genetic (a<sub>jt</sub>) and permanent environment (pe<sub>jt</sub>) were fitted for the t<sup>th</sup> milk production trait (y<sub>ijkt</sub>). Genetic parameters were obtained by using VCE (Variance Components Estimation) software based on the following mathematical model:

$$y_{ijkt} = (HYSopMol)_{it} + \left[ \sum_{R=1}^2 \beta_{Rt} * (A_{ijkt})^R \right] + a_{jt} + pe_{jt} + e_{ijkt}$$

**Results** Multivariate REML estimates of heritability as well as genetic and environmental correlations between monthly test day milk production traits are shown in Table 1. Generally, heritability of monthly test day milk yield was the highest followed by fat percentage and fat yield. Over the period of lactation, genetic correlation between monthly test day milk yield and fat percentage was negative while positive genetic correlations were found between monthly test day milk and fat yields and between monthly test day fat yield and fat percentage. The same pattern was also observed for environmental correlations between monthly test day milk production traits.

**Table 1** Estimates<sup>1</sup> of heritability (diagonal), genetic (upper diagonal) and environmental (lower diagonal) correlations between monthly test day milk production traits obtained from multivariate repeatability test day model

Trait	Milk yield	Fat yield	Fat percentage
Milk yield	<b>0.185 (0.020)</b>	+0.541 (0.067)	-0.694 (0.055)
Fat yield	+0.455 (0.004)	<b>0.072 (0.013)</b>	+0.212 (0.088)
Fat percentage	-0.314 (0.005)	+0.638 (0.003)	<b>0.119 (0.013)</b>

<sup>1</sup> Standard error of the estimate is into parenthesis

**Conclusion** The heritability estimates of monthly test day milk production traits of first parity cows obtained in this study are generally lower than those obtained by previous studies such as Strabel and Szwaczkowski (1997) and Rekaya *et al.* (1999). Lower heritability estimates of monthly test day milk production traits of Iranian Holstein cows could be attributed to several factors such as structure of the data and the model used for the analysis of the data. However, the main reason for lower heritability estimates in Iranian Holstein cows is due to rather low additive genetic and high environmental variation (consisting of permanent environment and residual variation) of monthly test day milk production traits as compared with the corresponding estimated variance components in other Holstein populations. The differences between the estimates obtained by others and the present ones could be partly explained by greater environmental changes in milk production which are mainly caused by different systems of feeding, keeping, housing and any other factors of environmental origin influencing performance of dairy cows in Iran.

**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 variation within and between five Iranian sheep populations using microsatellite markers

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**Introduction** In Iran, sheep is a main domestic animal with population about 60 millions. So, evaluation of genetic variation in Iranian sheep is a critical necessity. This study is the first research on genetic variation within and between a small part of Iranian sheep using microsatellite markers.

**Material and methods** Genetic variation at 6 microsatellite loci including McMA2, McMA26, MAF64, OarAE64, OarCP26 and OarFCB304 were analyzed for five Iranian sheep consisting of sanjabi (SAN), kordi kordistan (KKO), kordi khorasan (KKH), mehraban (MEH) and moghani (MOG). The number of DNA samples were 35, 32, 25, 25 and 24 for SAN, MOG, KKO, KKH and MEH, respectively. Genomic DNA was extracted by salting-out method (Miller *et al.*, 1988) with some modifications. All PCR reactions contained the following components: dNTPs (200 $\mu$ M), mgcl<sub>2</sub> (3.5mM), each of primers (0.25 $\mu$ M), *Taq* polymerase (1U/reaction), DNA (100-200ng/reaction). The products were electrophoresed on 8% nondenaturing polyacrylamide gels and bands visualised by rapid silver staining (Sanguinetti *et al.*, 1994). D<sub>A</sub> genetic distance (Nei *et al.*, 1983) was calculated and phylogenetic trees were constructed using both UPGMA and neighbor-joining. The unbiased average expected heterozygosity (H<sub>e</sub>) (Nei, 1978) as interpopulation variation were calculated. Various polymorphism criteria such as PIC values and number of alleles were estimated at studied loci and sheep. The time of divergence between two kordi populations (KKH and KKO) was estimated as Buchanan *et al.* (1994) have described.

**Results** PCR reactions were successfully done with all primers excepted to OarAE64. New alleles were found at Iranian sheep that haven't been previously reported. This shows Iranian sheep make an unique part of sheep genetic resources. Three All populations at all loci were in HWE excepted to MOG at McMA2 (p<0.005). Each locus per all populations was significantly deviated from HWE excepted to OarFCB304 (p<0.005). The lowest D<sub>A</sub> was between KKH and KKO (0.234) and also between SAN and KKO (0.246). These distances are rational due to co-descendant of two kordi sheep, short time passed from their separation (about 400 years old) and neighboring of their areas. Their phenotypic similarity confirm these distances too. The highest D<sub>A</sub> was between SAN and MOG (0.388). Due to long geographic distance and natural barriers (mountains and sea) between MOG and other populations, we considered MOG as an outgroup. So, it is expected to high genetic distance between MOG and others. Longtime use of MEH rams for crossing with other sheep may be occurred low distance between MEH and MOG and intermediate distance MEH with three populations. Both phylogenies (UPGMA and NJ) have two separate clusters. One includes KKO, KKH in a branch and then SAN. Another consists MEH and MOG. A possible explanation for this topology was expressed above. Interpopulation diversities were sufficiently high (0.506 to 0.915). Various polymorphism criteria indicated high polymorphism. The divergence time of two kordi populations (KKH and KKO) was estimated 445 years old. This time is very near historical evidences (about 3.5 years old).

**Conclusion** This research showed high variation within and between studied Iranian sheep populations and also proved that microsatellite genotyping is an useful tool to evaluating of variation and the evolutionary relationships among animal populations such as sheep.

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## Relationships among udder type traits and milk yield of Iranian Holstein-Friesian cattle

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**Introduction** Correlation among type traits and with milk production has been investigated by Brotherstone (1994) and Misztal et al (1992). One of the primary reasons for collecting and utilizing information on type traits is to aid breeders in selecting profitable functional cows for high production and suitable herd life. The objectives of this study were to estimate phenotypic and genetic correlations among milk production and with udder traits.

**Material and Methods** The production data on 4721 lactation records of 3250 cows of 5 herds at the suburb of the Tehran from 1992 to 1998 were retrieved from computerized data base of official milk yield (MY), fat yield (FY) and fat content (FC) of Cattle Breeding Center of Iran. A total of 783 cows received a linear score between 1 and 50 for each trait by using individual scorecard. The type traits considered were final score (FS), fore udder attachment (FUA), rear udder height (RUH), rear udder width (RUW), suspensory ligament (SL), teat placement rear view (TPR), and teat size (TS). Single and multiple trait animal models were used to estimate variance and covariance components of production and udder type traits by REML algorithm using DFREML program of Meyer (1997).

**Results** The least square mean of milk yield, fat yield and fat content were 6574±193.67, 208±6.66 Kg and %3.21±0.10 respectively. The least square mean of FS, FUA, RUH, RUW, SL, TPR and TS were 75.50±0.10, 16.90±0.28, 23.50±0.22, 29.80±0.22, 24.30±0.33, 16.61±0.25 and 26.49±0.23 respectively. The phenotypic and genetic correlations among MY, FY, FC and with udder type traits are given in Table 1. The phenotypic correlations estimated between MY and udder traits is ranged from -0.14 to 0.27 for FUA and RUW respectively that it whereas genetic correlations is ranged from -0.48 to 0.40 for FUA and RUW too.

**Table 1** Heritabilities, phenotypic and genetic correlations among production traits and with udder type traits

Traits	MY	FY	FC	FS	FUA	RUH	RUW	SL	TPR	TS
MY	<b>0.23</b>	0.50**	-0.45**	0.09 <sup>NS</sup>	-0.48**	0.30**	0.40**	-0.40*	0.01 <sup>NS</sup>	0.20*
FY	0.48**	<b>0.21</b>	0.62**	0.27**	-0.15*	0.25*	0.39**	-0.20*	0.04 <sup>NS</sup>	0.19*
FC	-0.32**	0.49*	<b>0.29</b>	-0.01*	0.15*	-0.18*	-0.10 <sup>S</sup>	0.13*	0.09 <sup>NS</sup>	-0.12*
FS	0.21**	0.23**	-0.01 <sup>NS</sup>	<b>0.19</b>	-0.50**	0.83**	0.98**	-0.73**	-0.02 <sup>S</sup>	0.90**
FUA	-0.14**	0.01*	0.10*	0.12*	<b>0.50</b>	-0.07	-0.76**	0.76**	0.35**	-0.12*
RUH	0.26**	0.14**	-0.11**	0.36*	-0.06*	<b>0.20</b>	0.72**	-0.31*	0.45**	0.30**
RUW	0.27**	0.20**	-0.05 <sup>NS</sup>	0.51*	-0.05*	0.60**	<b>0.33</b>	-0.76**	0.70**	0.25*
SL	-0.12**	-0.10**	0.07*	-0.74*	0.15**	0.01*	-0.08*	<b>0.23</b>	0.71**	-0.10*
TPR	0.05 <sup>NS</sup>	0.11*	0.05 <sup>NS</sup>	0.09 <sup>NS</sup>	0.07*	-0.17*	-0.06 <sup>S</sup>	0.28**	<b>0.10</b>	0.15*
TS	0.09*	0.07 <sup>NS</sup>	-0.09*	0.35*	0.09*	0.22**	0.20**	-0.10**	0.09 <sup>NS</sup>	<b>0.20</b>

Phenotypic correlations (below diagonal), genetic correlations (above diagonal) and heritabilities (on diagonal)  
 (\* P≤ 0.05    \*\* P≤ 0.01    NS = Non Significant)

**Conclusion** Genetic and Phenotypic correlations among milk yield and type traits shows that selection for udder type traits especially RUW and RUH could be effective in improving milk yield and also high emphasize on RUH and RUW traits for calculation of FS is recommended.

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## Ultrasonography as a predicting tool for carcass traits of young Nellore crossbred bulls in a feedlot system

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**Introduction:** Ultrasound technology provides a opportunity to quickly and economically estimate carcass attributes on the live animal (Brethour, 2000). In general, this technology has been used to detect variation for fat depth and ribeye area (*longissimus dorsi* muscle) in performance tested yearling bulls at several countries. In the present study, real time ultrasonography was used to predict the ribeye area (RA) and the subcutaneous fat thickness (FT) in Nellore crossbred.

**Material and Methods:** A total of 115 yearling bulls from four genetic groups (30 Angus x Nellore crossed, 30 Canchim x Nellore crossed, 30 Simmental x Nellore crossed and 25 Nellore), with 329 kg initial average weight, and two different finishing frame sizes (small and large) were used. Animals were kept in a feedlot during the whole study. Four ultrasonographic measurements were taken every 28 days until slaughter as Herring et al. (1994). Regression equations were used to identify the potential of ultrasound in predicting RA and FT in the live animals. RA and FT were measured in the carcasses and compared to the measurements made in the live animals.

**Results:** Predictive accuracy of the ultrasonographic measurements increased as the animals approached slaughter date, reaching the maximum value at the last one ( $R^2 = 0.68$  and  $0.82$  for RA and FT, respectively). Frame size did not influence RA or FT probably due to the small differences between the two groups. The predictive accuracy of FT ( $R^2 = 0.82$ ) was higher than Bullock et al. (1991) and Silva et al. (2001), and very similar of Perry & Fox (1997). Despite of the RA value ( $R^2 = 0.68$ ) had less accuracy, it showed similar behavior than FT. The smaller value of predictive accuracy for RA possibly was given by problems with transducers positions, carcass changes by *rigor mortis* or cleanness.

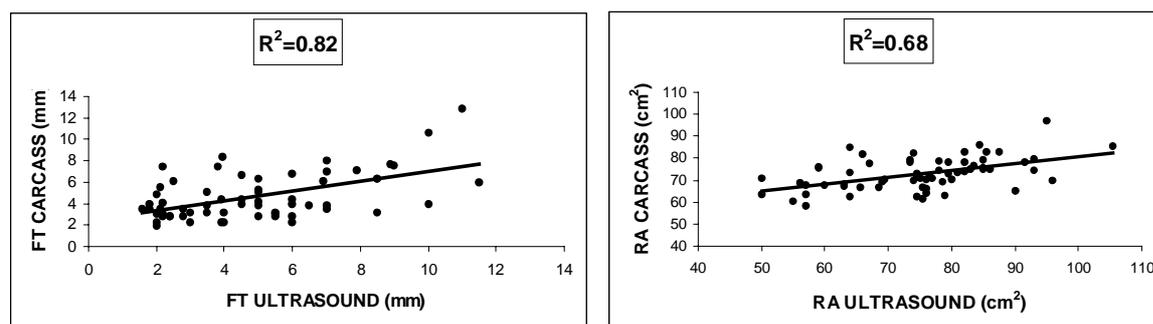


Figure 1 Comparison of ultrasound and carcass measures

**Conclusions:** This research further validates ultrasound as a useful tool for the prediction of fat thickness and ribeye area in young bulls. Since these traits are moderately heritable, ultrasound may reduce the need for progeny testing in a genetic improvement program for carcass traits.

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## Timing of ultrasonic scanning for Welsh Mountain rams

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**Introduction** Performance recording schemes for sheep flocks in the UK incorporate ultrasonic scanning to measure criteria that are linked to carcass lean content as a selection objective (Simm, 1998). In Wales, under the direction of the Welsh Sheep Strategy, fat and muscle depth has been monitored routinely in hill flocks but with a range of ram ages at scanning (Anderson, 2000; Ap Dewi, unpublished). The date of scanning is often dictated by timings of routine tasks within the flocks so there is a need to explore the impact of time of scanning on the measurements obtained. The objectives of the investigation were to study how muscle and fat depths changed over a series of scanning dates.

**Materials and Methods** Fat and muscle depths were obtained for 83 Welsh Mountain rams which were weighed and scanned on three dates, in October (6 months of age), December (8 months of age), and February (10 months of age). The scanning dates were 17<sup>th</sup> October 2000, 6<sup>th</sup> December 2000 and 7<sup>th</sup> February 2001. Throughout this period rams grazed outside at a lowland site without supplementary feed (Ap Dewi et al., 2002). An experienced operator from Signet carried out the ultrasonic scanning following their defined protocols (Signet, unpublished). Rank correlations were calculated for muscle and fat depth across traits and across each combination of the three recording dates. The repeatabilities of fat and muscle depths were estimated as the ratio of the variance due to animal and the sum of the animal variance component and residual variance. The variance components were estimated in a model that included animal and time and with animal as a random factor.

**Results** The mean fat depths were 4.43mm, 4.44mm and 4.17mm in October, December and February respectively. The muscle depths on these dates were 21.8mm, 22.1mm and 22.7mm respectively. Rank correlations for muscle depth and fat depth across the recording dates are shown in Table 1. The rank correlations for muscle depth and fat depth were all significant ( $P < 0.05$ ) with moderate to high correlations between recording dates. The repeatabilities of muscle and fat depth were 0.72 and 0.61 respectively.

**Table 1** Rank correlations (probabilities in parentheses) for ultrasonically measured muscle and fat depth across three dates

	Muscle Depth		Fat depth	
	December	February	December	February
October	0.81 (<0.001)	0.64 (<0.001)	0.68 (<0.001)	0.58 (<0.001)
December		0.69 (<0.001)		0.48 (<0.001)

**Conclusions** The results show that the ranking of animals were not significantly ( $P < 0.001$ ) affected by scanning date and both muscle depth and fat depth had moderate-high repeatabilities. It should be noted that the rank correlations across scanning dates for fat depths were consistently lower than those for muscle depth. The results suggest that ultrasonic scanning, as part of a flock recording programme, can be timed flexibly between 6-10 months of age. This represents the first winter of an animal's life. This allows an opportunity to scan at times that coincide with routine operations on farms. There is a need to extend the work to examine the repeatability of scanning results over a wider time period from 3 months (when lambs are normally weighed in hill flock recording schemes) to 18 months (mating). It would also be interesting to repeat the work with ewe lambs.

### Acknowledgements

Ultrasonic scanning was performed with the assistance of the Welsh Sheep Strategy

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## The use of 17 RAPD primers in some of Iranian sheep breeds

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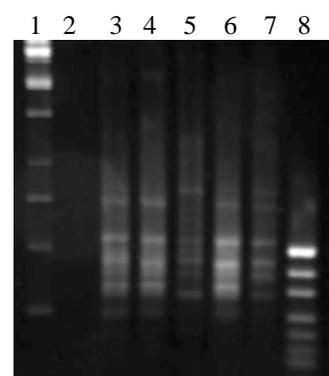
**Introduction** The randomly amplified polymorphic DNA technique was developed by Williams et al,(1990) and Welsh and McClelland et al.(1990). The technique is based on polymerase chain reaction using primers homologous to random target sites in the genome. The main advantages of RAPD assays are that it is simple, less labour intensive, comparatively less expensive and safe than other methods. The RAPD have been used for various applications including species identification, establishing genetic relationships, estimating genetic diversity and genome mapping in various livestock species including sheep. These studies reflected the effectiveness of rapd as potential genetic marker. The objective of the current study was to investigate RAPD marker development to distinguish genetic differences and similarity between and within some Iranian sheep breeds.

**Materials and Methods** Blood samples (5-10 ml) from 128 animals (13 Arman, 33 Balouchi, 10 Iranblack, 47 Kordi and 25 Karakol) were collected in EDTA-tubes. DNA was extracted from 100 µl of blood according to guanidiniumthiocyanate-silica gel procedure. All oligonucleotide primers were 10 to 16-mer (Table 1). Forty of this primers were reported by Kantanen et al (1995) that can produce repeatable bands in sheep and remainder three primers were chosen according to unpublished data. The PCR reactions (25 µl) contained 1 U Taq DNA polymerase; 2.5 µl dNTPs; 2.5 V Tris buffer (pH=8.8); 1.5 µl MgCl<sub>2</sub>; 10.3 µl dH<sub>2</sub>O; 2.5 µl glycerin; 50 ng of random primer and 20-100 ng of DNA. Following an initial denaturation step at 94 °C for 2 min, the reactions were subjected to 35 cycles of amplification at 94 °C (1 min), 36 °C (1 min) and 72 °C (2 min) with an additional extension step for 10 min. Amplification products were visualized by electrophoresis in 1.4 % agarose gels in 1x TBE buffer in the presence of ethidium bromid. The gels were photographed under UV light.

**Results** By using all primers, no differences were seen within breeds. Out of 17 primers only 3 primers( Moh-4, Moh-13 and Moh-21) created 10 polymorphic fragments. Primer Moh-4 produced seven bands in all breeds in overall that only 5 of them were seen in Arman breed and other breeds had all 7 bands. Primer Moh-13 created 11 bands that one band (1.5 Kbp) was absent in Balouchi and als in Kordi there was only 7 bands with this primer that 3 of them (1.1, 1.9 and 4.3 Kbp) were specific to this breed. With primer Moh-21, seven bands were amplified in all breeds that band 1 Kbp was not in Arman and Balouchi and another band (3 Kbp) was absent in Balouchi (figure 1).

**Table 1.** RAPD Primers used in this study

Name	Primer sequence	Name	Primer sequence
Moh-1	TGGACTCGAG	Moh-21	AACCGCGGTCT
Moh-2	GCACTGAGTA	Moh-23	TGCCAGTCTCC
Moh-4	GCATGCGATC	Moh-25	CTCAGGCTATGC
Moh-6	ACGTCGAGCA	Moh-26	CGAACCTGATC
Moh-7	TACGCAGACT	Moh-27	GCTTGCAGATC
Moh-11	TGCATCGTAC	P-1	CCGGCCTTAC
Moh-12	ACGCCGTACG	P-2	CACAATTCCACACAAC
Moh-13	GCTGCTCGAGT	P-3	GGGGGTTAGG
Moh-18	GCTAGCTACTG		



**Figure 1.** RAPD-PCR with primer Moh-21. Lane 1 is 1Kbp DNA ladder, lane 3-7 are samples from Karakol, Kordi, Balouchi, Iran-black and Arman respectively. Lane 8 is pUC19/MspI DNA.

**Conclusion** Results of the present study indicate that using this markers genetic diversity within and between sheep breeds are very low. As samples had been supplied from a research farm that had pure sheep stocks, high genetic similarity within breeds was expected. In fact according to the report of J. Kantanen et al (1995), sheep populations have high degree of homogeneity. More studies with more primers are needed to finding RAPD markers that are informative within and between Iranian sheep breeds.

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## Evaluation genetic parameters for weight at different ages in Baluchi breed of sheep

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**Introduction** Improving breeding values and breeding programs should be done based on genetic potential. The range of additive direct heritability and maternal environment heritability for birth weight is about 0.07 to 0.22 and 0.1 to 0.33 respectively the range of these values for the following weights are 0.09- 0.58 and 0.01- 0.17 .the objective of this study was to predict the direct additive genetic effect , maternal genetic effect and heritabilities of lamb weight traits in baluchi breed of sheep .

**Materials and methods** The studied traits in this experiment were birth weight (BW), three month weight (3W), six month weight (6W), nine month weight (9W) and yearling weight (YW) for prediction of genetic parameters. Characteristic of records are given in table 1 .Approximately 5913 records of Baluchi breed sheep from Abasabad sheep breeding station in north-east of Iran were used. Genetic parameter were estimated by using REML method under animal model. Different models of DFREML package were applied for this purpose. The best model for each trait was selected with likelihood ratio test . the best used models were model one ;  $y = Xb + Za + e$  and model two ;  $y = Xb + Za + Wp + e$  where y is a vector of observation ; b is a vector of fixed effect (included age of dam, sex of animal , birth type , year and month of birth); a is a vector of direct additive genetic effects ; p is a vector of common environmental effect due to ewe ; e is a vector of residual effect ; X , Z and W are matrices due to fixed and random effects. A number of days between birth date and the time of obtaining each record was used as a covariable for correcting the real data .

**Table 1** Characteristic of records

Character	BW	3W	6W	9W	YW
Mean (kg )	4.31	22.4	31.7	34.4	38.6
Standard deviation (kg)	5.68	4.4	5.6	5.7	6.3
Coefficient of variation ( % )	15.7	19.6	17.6	16.5	16.3
Number of record	5913	5146	4434	3671	3716
Number of fixed effect + covariable	4+1	5+1	4+1	4+1	4+1

**Results** Variance component and genetic parameter are presented in table 2. The best model for birth weight was model one and for other traits was model two. This study shows that the maternal environment genetic effect had a significant influence on growth traits in early age of lambs but in other ages direct additive effect was significantly important. Prediction genetic parameters for growth traits show that with increasing the age of lamb the direct additive heritability increases and maternal environmental component decreases .

**Table 2** Estimation genetic parameter

Period	$\sigma_p^2$	$\sigma_a^2$	$\sigma_c^2$	$\sigma_e^2$	$h_d^2$ (s.e)	$c^2$ (s.e)
BW	0.3	0.042	0.064	0.192	0.14 ( 0.032 )	0.21 (0.018)
3W	13.25	3.71	—	9.53	0.28 ( 0.045 )	—
6W	19.09	4.94	—	14.14	0.25 ( 0.05 )	—
9W	18.87	5.38	—	13.48	0.28 (0.053)	—
YW	23.84	7.96	—	15.8	0.33 ( 0.055 )	—

$\sigma_p^2$  = phenotypic variance;  $\sigma_a^2$ = direct additive genetic variance;  $\sigma_c^2$  = maternal environment genetic variance  
 $\sigma_e^2$  = residual effect variance;  $h_d^2$  = direct additive heritability;  $c^2$  = ratio of maternal environment variance to phenotypic variance

**Conclusions** The results of the present study demonstrate that direct additive genetic effect have significantly influences on three month weight and following age . Improving direct additive genetic effect has potential to improve maternal environment genetic effect .

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# Evaluation of the lamb and feed-lot performances of three crossbred and one purebred genotypes of Iranian fat-tailed sheep

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**Introduction** Sheep growers in Iran who raise breeds with light body weight, most often are interested to use rams of the heavier breeds to inseminate their ewes in order to obtain heavier lambs at sale. However, this practice may or may not be supported with enough scientific evaluations, on which only the results of very few studies are available (Kiyanzad, 2001; Farid, 1991; Makarechian *et al* 1978). The objective of this work was to declare the growth and feedlot performances of crossbred lambs, resulted from Afshari, Moghani and Shal (three heavy breeds) rams and Varamini (a light breed) ewes. The performance of pure Varamini lambs was also adopted as a comparison benchmark.

**Materials and methods** 188 Varamini ewes of the flock of the University Research Station were allocated to four groups (47 each). The ewes were randomly distributed within age groups of which we tried to have equality in each of four groups. Each group of ewes was exposed to three rams of one of the mentioned breeds for mating. The lambs, after weaning were divided into two almost equal groups including both sexes. The first group was fed a fattening diet for 15 weeks, while the second group was run on the crop remainder as its mother flock, and brought to feedlot after the first group. The data of number of lambs born, number of lambs weaned, birth weight (BW), weaning weight (WW), average daily gain from birth to weaning (ADG), feedlot final body weight (FFBW), feedlot average daily gain (FDG), feedlot dry matter intake (FDMI), feedlot feed conversion ratio (FFCR), warm carcass weight (WCW) and removable fat of the carcass (RFC) were collected and analyzed. Due to inequality in the number of observations at different subgroups, resulting data were analyzed using Generalized linear models (GLM) and least square means comparisons of SAS software.

**Results** The overall means for a number of traits are given in table 1. The known effects of sex, birth type, fattening period, etc. and their interactions with genetic group were taken into account for analysis of the data. Male lambs had significantly higher BW than females ( $p < 0.05$ ), But this difference for WW and ADG was not significant. Genetic group, sex and feedlot trial significantly ( $p < 0.01$ ) affected the FDG. The overall FDG of four genetic groups (1 to 4 respectively) were 177, 166, 169 and 159 grams, of which the difference between 1<sup>st</sup> and 4<sup>th</sup> is significant ( $P < 0.05$ ). The carcass of pure Varamini lambs had more removable fat including fat-tail than that of Shal  $\times$  Varamini lambs, but this difference was not significant with others. The overall results of this study showed a higher FDG and a better FFCR in second feedlot trial compare to the first ( $P < 0.05$ ).

**Table 1** The least square means<sup>#</sup> of genetic groups for different preweaning and feedlot traits<sup>\$</sup>

genetic group of lambs	BW (grams)	WW (kgrams)	FFCR		WCW(kgrams)		RFC(percent)	
			1 <sup>st</sup> period	2 <sup>nd</sup> period	1 <sup>st</sup> period	2 <sup>nd</sup> period	1 <sup>st</sup> period	2 <sup>nd</sup> period
1) Afshari $\times$ V*	3840 <sup>ab</sup>	17.80 <sup>ab</sup>	7.09	6.53	19.42	21.50	21.76 <sup>ab</sup>	23.94
2) Moghani $\times$ V*	3980 <sup>a</sup>	18.06 <sup>a</sup>	7.15	6.32	18.92	19.56	21.95 <sup>ab</sup>	22.64
3) Shal $\times$ V*	3940 <sup>a</sup>	17.76 <sup>ab</sup>	7.07	6.25	18.47	20.38	20.00 <sup>b</sup>	21.09
4) Varamini $\times$ V*	3700 <sup>b</sup>	16.82 <sup>b</sup>	7.24	6.98	18.96	20.02	23.38 <sup>a</sup>	22.07

#Different letters indicate significant ( $P < 0.05$ ) difference of means. \$Abbreviations are as defined in the text.

\* V = Varamini ewes

**Conclusions** Genetic group has significantly affected the preweaning traits, however, WCW and FFCR of the lighter pure Varamini weaned lambs were not significantly different from those of crossbred progenies. The starting time for feedlot operation seems to be independent of the lamb genotype and if the time for marketing lamb were not important, that starting time would be highly dependent on the costs of the rearing between weaning and feedlot period. Considering the traits which are more important as objectives, the crossbred lambs have perform better than the pure Varamini lambs, although some traits have not shown significant differences.

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## Inbreeding and its effects on some economic traits in Raeini cashmere goats

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**Introduction** The Breeding Center of Raeini (BCR) cashmere goats was established in 1965 in Kerman province, with a base population of 120, 8 and 42 does, bucks, and kids respectively. Some new animals have been introduced to the BCR population in some periods of time, and selected males have been sold out to the local breeders each year. Animals have been selected based on their phenotypic performance for fleece weight (FW) and fleece color (FC-white), and body weight (BW). Mating system has been planned based on non-relative mating, but some relative mating has been occurred. Inbreeding depression is one of the most important impact of having inbreeding in a population (Miglior and Burnside 1995). A decrease of 0.8% in fiber length and 6.3% in longevity per 10% increase of inbreeding coefficient in cashmere goats has been reported (Deb 1998). The objective of this study was to estimate the animals inbreeding coefficient and to explore the impact of inbreeding on some economic traits in Raeini cashmere goats.

**Material and Methods** Pedigree file included 10324 animals, which have been registered through years 1965 to 1999. Data file included 6598, 3509, 1815, 2120 and 4010 records for birth weight (BW), weaning weight (WW), six months weight (6MW), nine months weight (9MW), and greasy fleece weight (GFW) respectively. Animals inbreeding coefficient (IC) were estimated using Tier (1990) algorithm. Estimated inbreeding coefficients (EIC) were used as covariates in statistical models to analysis the performance records for mentioned traits. (Co) variance components were estimated using animal model and DFREML method with and without IC as covariates.

**Results** The estimated inbreeding coefficient ranged from 0 to 25.78% with the average of 0.276% . Most of animals (86.6%) were non-inbred (F=0) and only 13.4% of them were inbred with the IC average of 2.065% which ranged from 0 to 23.78%. The effect of animal inbreeding was significant ( $P<0.05$ ) on BW and 6 MW but non-significant on the other traits. Dams inbreeding coefficient affected WW and 6MW significantly, but had no significant effect on BW, 9MW, and GFW. The regression coefficient of BW, WW, 6MW, 9MW, and GFW on IC for the whole population, inbred animals, and inbred animals with inbred dams are given in Table 1.

**Table 1-** Regression coefficient of different traits performance (kg) on animals inbreeding coefficient

Animals	BW	WW	6MW	9MW	GFW
All	-0.00156	+0.0176	+0.0746	0.1506	0.071
Inbred	-0.00607	-0.0247	-0.0785	-0.467	-0.1034
Inbred with inbred dams	-0.0185	-0.0531	-0.1439	-0.0997	-0.139

Estimated heritabilities for BW, WW, 6MW, 9MW, and GFW were 0.224, 0.153, 0.222, 0.227 and 0.242 respectively from multivariate animal model. The genetic correlation coefficients between GFW and BW, WW, 6MW, 9MW were 0.238, 0.171, -0.012 and -0.142 respectively, and genetic correlation coefficients between body weights at different ages ranged from 0.222 to 0.899.

**Conclusions** The results of present study show that inbreeding is not a serious problem in the BCR goat population at present time. The performance of inbred animals has been slightly decreased for mentioned traits . Inbreeding trend could be fastened due to selection based on animal model BLUP evaluation which currently is going to practiced by BCR unless care is taken to restrict breeding.

**Acknowledgements** The Research Council of the University of Tehran for granting this research and the BCR for preparing data are acknowledged.

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## Differences in mammary gland weight and litter performance between Large White sows selected for different traits.

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**Introduction** Genetic selection has increased pig lean tissue growth rate, the most extreme animals comprising the 'sire' lines of breeding stock. However such improvement has not been without cost in other areas of production. Sire line sows are characterised by smaller litters with poorer pre-weaning growth rates than dam line sows of the same breed. The aim of this experiment was to determine whether reduced pre-weaning growth rate of sire line piglets was due to poor lactation performance of the sow or reduced vitality of sire line piglets.

**Materials and methods** Lactation performance of sire line Large White (LW) sows (S) was compared with that of dam line LW sows (D) when each sow pair had litters with the same proportion of S to D piglets. Sow performance was measured as piglet growth which is positively correlated to dam milk production (King *et al* 1997). Mammary gland weight was measured in a sample of sows to establish whether there were differences in secretory capacity between the two lines. 44 mixed parity LW sows comprising 22 S and 22 D sows farrowed in conventional farrowing crates. Parturition was induced by intramuscular injection of 2ml Illerin (Intervet). Feed was provided to appetite to sows and piglets did not have access to creep feed. Water was available to both sow and piglets *ad libitum*. Sows were matched according to parity. Litters were cross fostered between each pair of trial sows within 24 h of farrowing. Litters were standardised to 10 piglets, the same ratio of sire: dam line piglets per paired sows, piglets were allocated by live weight, sex and breed. Piglets were weighed on days 0 (start), 1, 2, 3, 7, 14 and 21 of the trial and at weaning (day 25.1± 0.22). Piglets consistently losing weight were weighed off trial and not replaced, as insufficient milk was available to support their maintenance and growth. Six S and six D sows were slaughtered within 2 h of weaning. The udder was removed and dissected. All skin, muscle, fat and connective tissue were removed leaving only the mammary secretory tissue. Each individual gland was separated and weighed. Only glands known to have been suckled at weaning were used in the subsequent comparison. Data was analysed using GLM and regression analysis procedures of Minitab 12.2.

**Results** By d3 of lactation piglets suckling S sows were already lighter than piglets suckling D sows (P<0.01, 1.99 vs. 2.09 ±0.019kg respectively). This reduced performance by S sows was maintained through to weaning with average piglet daily gains from d3 to d21 of 208 versus 240 g/pig/day for piglets reared by D sows (P<0.001). By d21 of lactation litter size of S sows was reduced to 7.55 whereas D sows still maintained 8.56 piglets per litter (P<0.001). This resulted in an estimated 2 kg per day less milk produced by S than by D sows. Gland weight per piglet tended to be lower for S sows (P<0.1). Both weaning weight and total weight gain per piglet were significantly related to gland weight. (P<0.001, R<sup>2</sup>= 0.19 and P<0.001, R<sup>2</sup>=0.34 respectively). Piglet genotype did not affect performance regardless of sow genotype suckled, daily weight gains to d21 were 223 and 219 (±3.5) g/d and d21 weights 6.27 and 6.15± 0.076 kg for D and S piglets, respectively.

**Table 1** Liveweight on d0, 3, 21 and at weaning, daily gain to d21, litter size and mammary gland weight for piglets suckling D and S sows and daily gain to d21 for D and S piglets regardless of sow genotype suckled.

	d0 pig wt (kg)	d3 pig wt (kg)	d21 pig wt (kg)	weaning wt (kg)	mean daily weight gain (g/d) to d21	d21 litter size	gland weight (g)	daily weight gain to d21 (g/d) for Piglet genotype
Dam line	1.62	2.09	6.42	7.76	227	8.559	695.3	223
Sire line	1.62	1.99	5.72	6.43	194	7.551	628.9	219
SE	0.013	0.019	0.076	0.170	3.5	0.106	18.8	3.5
Significance	ns	P<0.01	P<0.001	P<0.001	P<0.001	P<0.001	P=0.093	ns

**Conclusions** Sire line sow lactation performance compromised piglet survival and growth rate when similar litters were reared on both S and D sows indicating that S sows have a reduced ability to secrete milk compared to D sows. This was at least in part explained by reduced secretory cell capacity as reflected in mammary gland weight. Piglet genotype did not affect performance indicating that S piglets had similar vitality to D piglets. Further work is necessary to establish what other factors may be involved in reducing sire line sow milking ability.

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# The use of fresh or thawed rumen fluid containing glycerol or particle associated microbes to estimate *in vitro* degradation of feeds

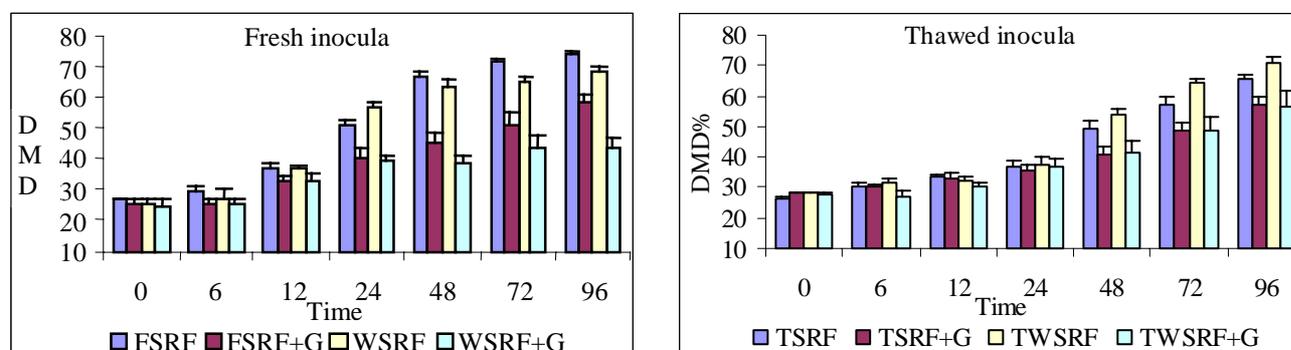
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**Introduction** Thawed rumen contents have been used to obtain strained rumen fluid (SRF) to estimate *in vitro* dry matter degradation (DMD) of feeds (Mohamed *et al.*, 2002). However, thawed SRF (TSRF) gave lower DMD than fresh SRF (FSRF) which was partly attributed to reduced microbial activity in TRSF following storage at -20°C. This study examined the addition of glycerol (G) as cryopreservative and washing from particle associated microbes to SRF before its storage for later use as TSRF to estimate *in vitro* degradation of rapeseed meal (Rsd) and grass nut (Gnt).

**Material and methods** Whole rumen contents (WRC) were obtained from cattle, one freshly slaughtered on each of three separate occasions, and were equally divided into WRC1 & WRC2. WRC1 was strained through cheesecloth to obtain SRF which was mixed with a buffer to prepare FSRF. One half was kept as FSRF and to other half G (5%) was added (FSRF+G). WRC2 was squeezed through cheesecloth to obtain another SRF. The solid residue was washed four times with four volumes of a pre-warmed buffer (pH 7.4) and the washings were then mixed with one volume of SRF to prepare washed SRF (WSRF). One half was kept as WSRF and to other half G was added to get WSRF+G. Each volume of FSRF, FSRF+G, WSRF and WSRF+G was separately divided into two halves. One half of each inocula was used fresh and the second half was stored at -20°C until used as thawed inocula (TSRF, TSRF+G, TWSRF and TWSRF+G). The study was conducted as a 2×2×2×2×3×7 factorial design in duplicate and involved 2 feeds (Rsd & Gnt), 2 inocula types (unwashed & washed), with or without G, fresh or thawed, 3 cows and 7 times (0, 6, 12, 24, 48, 72, 96 h). About 0.4g of a feed (<1mm) was weighed into a test tube to which 40ml of buffered inoculum were added, flushed with CO<sub>2</sub>, capped and incubated at 39°C for various times. At the end of each time, the residues were washed, dried and weighed to estimate DMD. Blanks were also run to correct DMD. The data were statistically analysed to study the main effects of washing, glycerol, thawing, time and their interactions and significance was declared if P<0.05.

**Results** Mean (±s.e) *in vitro* DMD (%) for each inocula (n=12) at each time, averaged over cows and feeds, and for each feed (n=42), averaged over cows and times, are shown in Figure 1 and Table 1 respectively. All main effect were significant (P<0.05, s.e.=0.41) except that for washing (P>0.05). Unwashed tended to give higher DMD than washed inocula (43.2 v 42.2). Thawed inocula produced lower DMD than fresh inocula (41.5 v 43.9). Inocula with glycerol showed lower DMD than that without glycerol (38.4 v 47). Individual comparisons showed the highest DMD for FSRF than all other inocula (Table 1). TWSRF produced the highest DMD compared to all other thawed inocula whereas TWSRF+G tended to produce higher DMD than WSRF+G. The overall mean DMD for Rsd and Gnt of this study match well with the DMD for the same feeds in our previous work using comparable inocula (Mohamed *et al.* 2002).



Feed (s.e.)	Unwashed				Washed			
	FSRF	TSRF	FSRF+G	TSRF+G	WSRF	TWSRF	WSRF+G	TWSRF+G
Rsd (1.1)	53.3 <sup>a</sup>	45.7 <sup>cd</sup>	42.5 <sup>de</sup>	43.2 <sup>de</sup>	50.8 <sup>ab</sup>	48.1 <sup>bc</sup>	41.4 <sup>e</sup>	44.8 <sup>cd</sup>
Gnt (0.97)	48.4 <sup>a</sup>	39.7 <sup>c</sup>	37.8 <sup>cd</sup>	35.1 <sup>d</sup>	46.9 <sup>a</sup>	43.1 <sup>b</sup>	30.1 <sup>e</sup>	32.1 <sup>e</sup>
Average (0.8)	50.9 <sup>a</sup>	42.7 <sup>c</sup>	40.2 <sup>cd</sup>	39.1 <sup>d</sup>	48.8 <sup>a</sup>	45.6 <sup>b</sup>	35.7 <sup>e</sup>	38.5 <sup>de</sup>

Means with different superscripts in the same row differ significantly (P<0.05).

**Conclusion** Addition of particle associated microbes to SRF had a positive effect on thawed inocula when glycerol was not added to SRF. However, the DMD values for thawed inocula were still lower than those for fresh inocula. The time required to obtain washings may limit the application of this procedure to prepare SRF. Addition of glycerol was unable to improve the DMD of feeds by thawed inocula and in fact showed reduction in DMD when fresh SRF was used instead of thawed SRF to incubate feeds. Further studies are continued to develop and improve *in vitro* methods to estimate degradation of ruminant feeds.

**Acknowledgement** to Mike Hearn for his assistance during this laboratory work.

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## The relationship between diet and the chemical composition of sheep faeces

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**Introduction** The use of faecal inoculum in *in vitro* feed evaluation methods was examined by Balfe (1985). However, there is limited information concerning the chemical composition of faeces and factors affecting this. The chemical composition of faeces may reflect the microbial population and hence its fermentative activity. A knowledge of the faecal composition is essential as this affects the quality of faecal inoculum. The objective of this work was therefore to study the relationships between diet and the chemical composition of faeces using data obtained from sheep.

**Materials and methods** The data used were from 471 digestibility studies done at ADAS Nutritional Sciences Research Unit from 1978 to 1999. Most experiments used four sheep but some used two or three sheep. There were 23 types of diet and the number of experiments per diet type ranged from 12 to 64. Diets consisted of both mixed and single component feeds. The mixed diets included palm kernel meal, feather meal, cotton seed meal, distillers' dark grains wheat, malt culms, fishmeal, brewers' grains, rape seed meal, prairie meal, untreated spring barley straw, untreated wheat straw, maize gluten feed, fresh sugar beet pulp and fresh grass. The single component diets included Rowett diet A (Rowett Research Institute, 1976), whole crop wheat, fresh grass, dried grass nuts, dried lucerne nuts, lucerne silage, grass silage (big bale and clamp) and maize silage. The data were used to investigate the relationships between the dry matter (DM), organic matter (OM), ash, crude protein (CP) and ether extract (EE) contents of faeces and the content and dietary intake of DM, ash, OM, CP, EE, NDF and water soluble carbohydrate (WSC). The mean values of each set of experimental data were statistically analysed for the relationship between faecal composition and the diet intake and its chemical composition using Minitab (1994). Matrix correlation was first applied to determine the overall association, if any, between the diet chemical contents and intake (DM, ash, OM, CP, EE, NDF and WSC) and the faecal chemical content (DM, OM, Ash, CP and EE). Subsequently, both monovariant and stepwise multiple regressions were applied to develop relationships between key parameters. Stepwise multiple regressions were arranged between each parameter response and the predictors. All associated predictors were included in the first step and variables dropped from the model if their F ratio was less than 4.0.

**Results** The content of CP in faeces (FCP) was positively correlated to the content of CP in the diet (DCP) and intake (ICP) per day ( $r = 0.680$  and  $0.597$  respectively) but negatively correlated to the content of NDF in diet (DNDF) and intake (INDF) per day ( $r = -0.428$  and  $-0.363$  respectively). The content of OM in faeces (FOM) was positively correlated to the content of EE in diet (DEE) and intake (IEE) per day ( $r = 0.376$  and  $0.385$  respectively). FCP was significantly ( $P < 0.001$ ) related to DCP and DNDF in the diet, ICP and INDF with  $R^2$  values of 45.6, 17.3, 34.8 and 12.1 % respectively (Table 1). FOM was also significantly ( $P < 0.001$ ) related to DEE and IEE with  $R^2$  13.8 and 13.1 % respectively (Table 1).

**Table 1** Regression equations between faecal (Y) and dietary composition and the total diet intake (X)

Variables	Equation	$R^2$ (%)	P
FCP $\times$ DCP	$Y = 58.7 + 0.569 X$	45.6	$< 0.001$
FCP $\times$ DNDF	$Y = 324 - 0.273 X$	17.3	$< 0.001$
FCP $\times$ ICP	$Y = 71 + 0.585 X$	34.8	$< 0.001$
FCP $\times$ INDF	$Y = 270 - 0.207 X$	12.1	$< 0.001$
FOM $\times$ IEE	$Y = 782 + 2.01 X$	13.8	$< 0.001$
FOM $\times$ DEE	$Y = 780 - 1.79 X$	13.1	$< 0.001$

**Conclusions** None of the relationships accounted for a high proportion of the variability in faecal composition suggesting that diet composition has only a limited effect on the composition of faeces. FCP was mainly affected by dietary concentrations and intakes of CP and NDF, with CP having a positive effect and NDF a negative effect. FOM was affected slightly by with EE intake. Since FCP and FOM might reflect the microbial numbers in faeces, this information is essential for the development of the use of faecal inocula in *in vitro* feed evaluation. It would seem however that more direct measures of hydrolytic and fermentative activity in faeces are required.

**Acknowledgements** The authors acknowledge ADAS NSRU for providing the data.

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# Relationship between rumen odd and branched chain fatty acids and fermentation characteristics as studied *in vitro*

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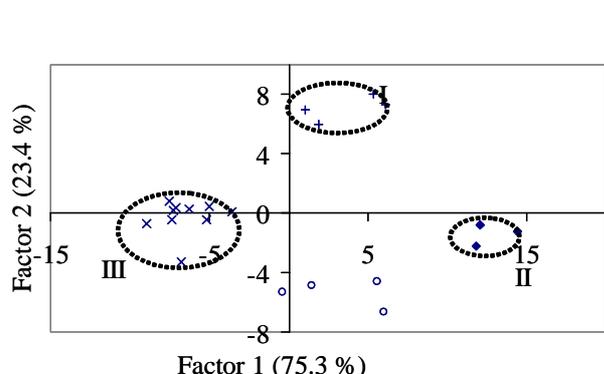
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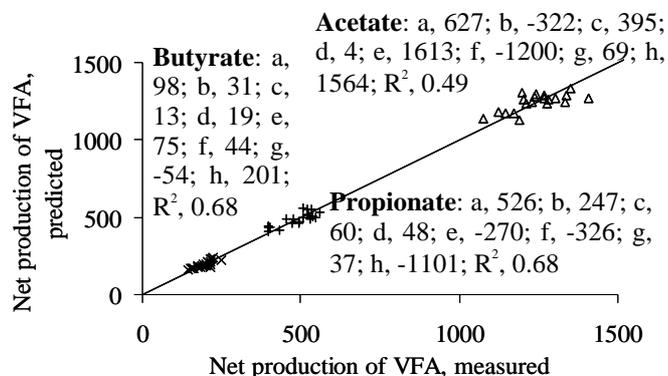
**Introduction** Rumen microbes contain a high proportion (20 to 50%) of their fatty acids (FA) as odd and branched chain fatty acids (OBCFA; C15:0, iso C15:0, anteiso C15:0, C17:0; iso C17:0; anteiso C17:0 and C17:1) and different bacterial classes have distinctive OBCFA 'fingerprints'. As OBCFA make up around 5% of FA in milk, it has been suggested that there is scope for these compounds to be used in on-farm diagnostic milk-based tests in relation to the rumen fermentation pattern. Correlations of milk OBCFA with rumen fermentation pattern were recently shown (Vlaeminck et al., 2002). In the current *in vitro* study, the potential of rumen OBCFA to predict the production of volatile fatty acids (VFA) was evaluated.

**Materials and methods** Eleven whole dairy cow diets (450 mg) were simulated using 3 types of grass silage (GS), varying in total tract digestibility of OM (72 to 80 %), 5 types of concentrates (Conc), mainly differing in level of rapidly fermentable carbohydrates (starch, from 81 to 181 g/kg DM) and 3 maize silages (MS), differing in DM content (26 to 36 %). Diets 1 to 4 consisted of GS and Conc (60/40, w/w DM), whereas diets 5 to 11 contained GS, MS and Conc (30/30/40 for diets 5 to 9, 25/25/50 for diet 10 and 10/10/80 for diet 11, w/w/w DM). Mixed rumen contents were obtained two hours after the morning feeding from two rumen fistulated sheep, fed twice daily at maintenance a hay/grain based concentrate (65/35, w/w DM). Incubations, carried out in duplicate, during 21 h and analysis of VFA, CH<sub>4</sub>, H<sub>2</sub> and higher FA, including OBCFA, were as described by Fievez *et al.* (2002). OBCFA and net VFA and CH<sub>4</sub> productions were analysed using Canonical Discriminant Analysis (DA) and Cluster Analysis (CA). OBCFA were also used in Principal Component Analysis (PCA).

**Results** Grouping of diets by DA and CA were similar when based on OBCFA (illustrated in Figure 1) and fermentation characteristics (data not shown), identifying group I containing diets 10 and 11, group II with diets 2 and 3 and group III with diets 5, 6, 7, 8 and 9. Based on OBCFA, DA and CA grouped diet 1 with diet 4 (Figure 1), whereas analysis based on fermentation characteristics linked diet 1 with diets 2 and 3. PCA was then used to create orthogonal contrasts in OBCFA. The relation of OBCFA with the rumen fermentation pattern was evaluated by multiple linear regression of individual fermentation characteristics on the principal component scores. Non significant component scores were removed from the regression. The predicted values from the regressions for net production of acetate, propionate and butyrate are shown relative to the measured values in Figure 2.



**Figure 1** DA based on OBCFA (I with diets 10 & 11; II with diets 2 & 3; III with diets 5, 6, 7, 8 & 9; diets 1 and 4 symbolised by O).



**Figure 2** Comparison of predicted (a+b\*isoC15:0+c\*anteisoC15:0+d\*C15:0+e\*isoC17:0+f\*anteisoC17:0+g\*C17:0+h\*C17:1) and measured values of net production of acetate ( $\Delta$ ), propionate (+) and butyrate (x) ( $\mu$ moles/incubation).

**Conclusions** The similarity of groups identified by DA and CA based on the rumen fermentation pattern and OBCFA gives scope for the prediction of the rumen fermentation pattern based on OBCFA, which is confirmed by the correlation of individual VFA with OBCFA. However, since this was an *in vitro* experiment, further research is needed to validate current findings *in vivo*, using milk OBCFA

**Acknowledgements** B. Vlaeminck is supported by a grant from the Flemish Institute for the Promotion of Scientific-Technological Research.

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# Prediction of chemical composition of maize silage by near infrared reflectance spectroscopy in Uruguay

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**Introduction** Whole-plant maize silage forms the basis of winter rations for the vast majority of dairy and beef cattle production in Uruguay. Microbiological examination of silage is of little value in gauging the outcome of silage, and so chemical analysis is more reliable and meaningful indicator of quality. Chemical assessments of the principal fermentation products provide an unequivocal basis on which to judge quality. Silage fermentation and chemical composition are important to preservation of forage with respect of feeding value and animal performance. Many of the important chemical components of silage must be assayed in fresh (wet presentation) or by extraction of the fresh material, since drying either by heat or lyophilisation, volatilises components such as acids or nitrogenous components, or effects conversion to other compounds (fibre and carbohydrates) (Abrams *et al.*, 1987). Chemical and biological methods for assess maize silage quality are laborious and considered to slow to be used for routine analysis of large number of forage samples. Near infrared reflectance spectroscopy (NIRS) is increasingly used as a rapid, accurate method of evaluating chemical constituents in cereals and dried forages. The objective of this study was to determine the potential of NIRS to assess the chemical composition of dried maize silage samples for advisory purposes.

**Materials and Methods** Three hundred and eighty (n = 380) whole-plant maize silage samples (*Zea mays*, L.) were collected from different locations, growing years (1997, 1998, 1999 and 2000), varieties and silo types in Uruguay and analysed at INIA La Estanzuela, Animal Nutrition laboratory (South America). Extracts of fresh silage were analysed for pH using a glass – electrode pH meter (Orion, Model 230 A, Orion Research, USA). Samples were dried at 65 °C for 48 hours to determine dry matter content (DM) and milled using a Wiley forage mill fitted with a 1mm mesh (Arthur H. Thomas, Philadelphia, PA, USA), before chemical analysis. Crude protein (CP) was calculated as the percentage of nitrogen x 6.25 (AOAC, 1990). Ash was determined by incinerating the samples at 500 °C for 16 hours (AOAC, 1990). Acid detergent fibre (ADF) was analysed following the Goering and Van Soest (1970) method. All chemical analysis was expressed on a dry weight basis. Dry samples were scanned in a small ring cup (50mm diameter) in reflectance mode (400 - 2500nm) (NIRSystems, USA). Reflected energy readings were referenced to corresponding readings from a ceramic disk. The spectrum of each sample was the average of 32 successive scans. Predictive equations were developed using modified partial least squares (MPLS) regression with internal cross-validation and scatter correction using standard normal variate (SNV) and detrend (Barnes *et al.*, 1989). Calibration statistics calculated include the standard error of calibration (SEC), the coefficient of determination in calibration ( $R^2_{CAL}$ ), the standard error of cross validation (SECV) and the coefficient of determination in cross validation ( $R^2_{VAL}$ ) (Shenk & Westerhaus, 1993). The optimum calibrations were selected based on minimising the standard error of cross validation (SECV).

**Results** Table 1 shows the NIRS calibrations statistics for the whole-plant maize samples on dry presentation.  $R^2_{CAL}$  coefficients and standard error in cross validation (SECV) were  $R^2$  0.91 (SECV: 22.1), 0.90 (SECV: 4.9), 0.93 (SECV: 57.1), 0.90 (SECV: 2.9) for dry matter (DM), crude protein (CP), acid detergent fiber (ADF) and ash in g kg<sup>-1</sup> dry weight, respectively. Near infrared calibrations for pH showed the lowest statistics.

**Table 1** NIRS calibration statistics for chemical parameters in dry maize silage (g kg<sup>-1</sup> DM)

	N	Mean	Range	SD	$R^2_{CAL}$	SEC
DM	320	346	234 - 528	68.7	0.91	19.3
CP	340	70.7	37.5 - 95.2	13.2	0.90	4.1
ADF	325	290	195 - 413	43	0.93	20.9
Ash	320	59.8	38.5 - 81.1	8.2	0.90	2.4
PH	332	3.5	3.2 - 3.9	0.2	0.82	0.1

**Conclusions** NIRS showed the capability to be used as a tool to predict chemical composition in whole maize silage for advisory purposes. Fresh presentation to the instrument will be explored in the future.

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# The use of near infrared reflectance spectroscopy (NIRS) for the prediction of the digestible energy content of barley for growing pigs

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**Introduction** It is not possible to carry out *in vivo* pig digestibility studies on each feed or feed ingredient therefore there is a need for a rapid means of predicting the digestible energy content of a feed or feed ingredient. Near infrared reflectance spectroscopy (NIRS) is an extremely rapid technique and has been used to predict chemical composition and nutritive value for a wide range of feeds and feed ingredients (Leeson *et al* 2000). In the literature, some workers have reported that NIRS has a high degree of accuracy for determining chemical composition and nutritive value while others have reported a lower degree of accuracy. The aim of the current study was to examine the value of NIRS in predicting the digestible energy (DE) content of barley from which pig diets were formulated.

**Materials and methods** As NIRS requires calibration with *in vivo* data, barley (n=39) from which pig diets had been formulated and values for DE determined, were used in this study (McCann 2001). The samples were scanned in duplicate using a Foss NIRSystem 6500 instrument and the spectra recorded as log 1 / R (reflectance). Mathematical treatment of the spectral data was performed using ISI – NIRS version 4.0 software and calibrations developed for DE content using the modified partial least squares (MPLS) regression technique. Transformations of the spectral data through derivatization and scatter correction procedures were examined and equations produced using the log1/R, first and second derivatized data. The mathematical derivatization of 1,4,4,1 was used in the first order and 2,10,5,1 was used in the second. The scatter correction programmes included were; WMSC (weighed multiplicative scatter correction), SNVD (standard normal variate and detrend) and NMSC (normal multiplicative scatter correction). Cross validation was undertaken using the standard methodology in the NIRS version 4.0 software programme. Optimum calibrations were selected as those with the lowest SECV (standard error of cross validation)

**Results** Table 1 shows the calibration and validation statistics for DE. DE values ranged from 14.4 to 16.4 MJ/kg DM. The 1,4,4,1 derivative combined with SNVD gave the lowest SEC (0.16) and the relationship for the calibration set was strong ( $R^2 = 0.91$ ). With cross validation, the SECV increased to 0.28 and the relationship was lower but still strong ( $R^2 = 0.70$ ). This indicates that the calibration was not particularly robust.

**Table 1.** Calibration and validation statistics for the prediction of DE (MJ/kg DM) content of barley using MPLS

Derivative	Treatment	Range	Mean	SEC	R <sup>2</sup>	SECV	1 - VR
1,4,4,1	WMSC	14.4-16.6	15.5	0.128	0.933	0.277	0.686
1,4,4,1	SNVD		15.5	0.157	0.908	0.282	0.700
2,10,5,1	SNVD		15.5	0.188	0.870	0.297	0.673
2,10,5,1	NMSC		15.5	0.188	0.871	0.299	0.669
2,10,5,1	WMSC		15.5	0.241	0.783	0.312	0.634
1,4,4,1	NMSC		15.5	0.166	0.899	0.322	0.618

SEC – Standard error of calibration, SECV – Standard error of cross validation, 1-VR – R<sup>2</sup> for cross validation

**Conclusions** The correlations derived by MPLS for DE value of barley were relatively strong. Strong correlations, based on calibration alone have been reported, however correlations based on validation sets are weaker and have higher standard errors than those for calibration (George 2000). This trend was observed to a certain extent although the correlation for validations remained strong and the standard error low. This may be attributable to the relatively small sample set. The results of this study suggest that NIRS has the potential to predict the DE of feed ingredients used in diets provided a large dataset is used for calibration.

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# Determination of dry matter (DM) and nitrogen (N) degradability in forages by near infrared reflectance spectroscopy (NIRS)

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**Introduction** The nutritive value of forage crops is related mainly to climatic conditions and stage of plant maturity, and its determination for any given crop is essential for optimum planning and animal feeding (Berardo et al., 1993; Deaville and Flinn, 2000). Worldwide the nutritive value of forages is often estimated by chemical or physical methods and is expressed as the concentration of chemical constituents in the plant tissue. There is little information in the literature about the use of NIRS to determine degradability in pastures with different conditions, season, different places (Wilman et al., 2000). The aim of the work to explore the use of NIRS as rapid tool for estimate DM and N degradability in forages.

**Materials and Methods** Seven pastures which have been assigned for silage making by the farmers composed by mixtures of grasses and legumes (*Festuca arundinacea*, *Lolium multiflorum*, *Trifolium repens*, *Trifolium pratense*, *Medicago sativa*) were used. Each pasture was sampling during each of the followings steps during the silage making: 1) fresh forage (F); 2) wilted forage (W) and 3) silage (S). Silages were made as nylon packed bales of approximately 700 kg. Samples of F were collected immediately after cutting from six different sites of the pasture. W was sampled after a field –wilted period that was determined by the farmer. After 60 days of ensiling samples of S were collected by sampling different bales for each forage. All forage samples were freeze-dried and ground to pass a 2-mm screen for use in degradability trials and to pass 1-mm screen for chemical analysis. Samples were dried in a forced oven at 60 °C to constant weight for 48 hours, and ground in a Wiley forage mill to pass a 1 mm screen (Arthur H. Thomas, Philadelphia, PA, USA). Nitrogen was determined using a semi – micro automated Kjeldhal method (Tecator, Sweden) and converted to CP using the factor 6.25 (AOAC, 1990). All chemical analysis was expressed on a dry weight basis and analysed in duplicate. Approximately 20 g of residue (n = 190) from the degradability trials was scanned, dry, in the visible and near infrared region of reflectance (400 – 2500 nm) in a scanning monochromator NIRS 6500 (NIRSystems, Silver Spring, MD, USA) in a small circular quartz cup (50 mm diameter) sealed with disposal paper. Samples were scanned once. Reflectance data were stored as log (1/R) at 2 nm intervals (where R is reflectance). Predictive equations were developed using modified partial least squares (MPLS) (Shenk and Westerhaus, 1993) regression with internal cross-validation (NIRS 2, 1995) and scatter correction using standard normal variate transformation (SNV) and detrending (Barnes et al., 1989). The optimum calibrations were selected based on minimising the standard error of cross validation (s.e.c.v.).

**Results** Table 1 presents the NIRS calibration and cross validation statistics for DM and N degradability in forage samples. The best r<sup>2</sup> and s.e.c.v were 0.98 (19.1) and 0.97 (19) for DM and N, using None and 1,4,4,1 as mathematical treatment, respectively. The s.e.c.v. values obtained in this study were according with those found for other authors. Using NIRS technique, the accuracy of prediction of DM and N degradability on forages is relatively similar to that reported by other authors. Good NIRS calibration statistics were obtained for DM and N respectively on forage residues.

**Table 1** NIRS calibration statistics for total DM and N degradability (g kg<sup>-1</sup> DM)

	n	Mean	SD	R <sup>2</sup> <sub>CAL</sub>	SEC	SECV
<b>N</b>						
None 1,4,4,1	180	691.0	129	0.97	19.0	25.0
None 2,5,5,2	180	697.5	130	0.97	21.0	27.0
SNVD 1,4,4,1	180	696.3	129	0.97	22.0	28.1
SNVD 2,5,5,2	180	696.4	131	0.97	22.0	28.2
<b>DM</b>						
None 1,4,4,1	182	513.1	162	0.98	19.1	24.1
None 2,5,5,2	183	516.8	163	0.98	22.1	27.1
SNVD 1,4,4,1	180	514.3	162	0.98	19.5	23.2
SNVD 2,5,5,2	182	515.9	162	0.98	20.0	26.2

SD: standard deviation, SEC: standard error in calibration, SECV: standard error in cross validation, n: number of samples used for calibration development

**Conclusions** NIRS shows the capability to be used as a tool to estimate DM and N degradability in forages.

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# The possible use of n-alkanes, long-chain fatty alcohols and long-chain fatty acids as markers in studies of the botanical composition of the diet of free-ranging herbivores

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**Introduction** Few methods exist for quantitatively estimating the diet composition of free-ranging herbivores. Because their patterns differ between species, plant n-alkanes have been successfully used as faecal markers to estimate the botanical composition of simple diets, however, the reliability of this methods may decline as the number of dietary plant species increases (Dove and Mayes, 1996). The objective of this study was to examine whether additional plant-wax compounds, such as long-chain fatty alcohols (Alc) or long-chain fatty acids (Ac) could be used along with n-alkanes (Alk) to allow reliable diet composition estimates to be made in herbivores consuming complex diets.

**Materials and Methods** Twelve Scottish Blackface wether sheep, weighing 40-45 kg and housed in metabolism crates were fed four different mixtures of three plant species (three animals per mixture) for a seven-period trial. These mixtures were M1=*Pinus sylvestris* (Ps)+*Lolium perenne* (Lp)+*Calluna vulgaris* (Cv), M2=*Picea sitchensis* (Psi)+ *Chamaenerion angustifolium* (Ca)+*Luzula sylvatica* (Ls), M3=*Pseudotsuga menziesi* (Pm)+*Fagus sylvatica* (Fs)+ *Vaccinium myrtillus* (Vm), M4=*Brassica oleracea* (Bo)+*Acer pseudoplatanus* (Ap)+*Juncus effusus* (Je). In each 7-day experimental period the diet (1.5 kg DM/d) was offered as two feeds and refusals collected daily; faeces were collected on the last day. Freeze-dried samples of feed and faeces were analysed for Alk, Alc and Ac using a modification of the alkane method of Mayes et al., (1986), which enabled these compound classes to be isolated from a single sample. The dietary proportions of each species were calculated by a least-squares optimization procedure (Hameleers & Mayes, 1998) using Microsoft Excel (solver). To explore the differences between the different marker methods, the mean squares of errors (EMS) between the actual and calculated diet proportions of plant species were calculated.

**Results** Except for the secondary alcohol 10-nonacosanol (10-C<sub>29</sub>-ol) which was the main alcohol in the conifer species (Ps, Psi & Pm), Alc and Ac with even-chain lengths predominated over the odd-chained compounds. The concentrations of the main Alc and Ac are shown in Table 1. The patterns of these compounds differed greatly between plant species.

**Table 1** Concentrations (mg/kg DM) of the prevailing even-chain alcohols and acids in various plant species

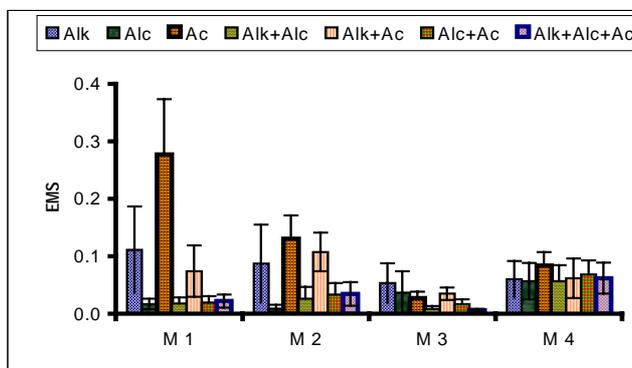
Feed	C24 Alc	C26 Alc	10-C <sub>29</sub> -ol	C28 Alc	C30 Alc	C24 Ac	C26 Ac	C28 Ac	C30 Ac	C32 Ac
Je	19.3	44.8	7.1	10.6	234.3	134.1	50.8	44.2	126.5	38.6
Ca	3860.7	518.0	4.6	119.1	89.5	134.4	106.9	96.3	52.2	4.5
Fs	191.1	88.8	7.0	3.3	38.9	111.2	71.1	291.8	15.7	6.9
Vm	285.3	248.3	7.5	167.5	602.6	139.6	128.4	324.8	1131.8	176.1
Cv	363.2	166.6	13.9	260.3	297.8	254.6	195.2	199.3	167.8	5.8
Ls	209.4	80.6	10.9	16.3	124.8	369.1	228.2	198.6	185.9	51.2
Ap	929.3	706.1	5.9	138.5	467.5	292.6	202.9	421.2	278.4	23.1
Ps	80.6	57.5	1853.3	13.0	114.0	159.0	21.0	31.9	63.4	36.4
Psi	84.7	17.9	2065.0	13.7	19.5	430.3	60.1	48.1	110.1	30.6
Pm	48.2	12.0	1591.4	30.7	26.0	220.4	37.1	49.6	356.5	120.6
Lp	104.0	2628.1	10.1	445.5	627.4	174.0	156.1	158.6	88.2	33.5
Bo	23.3	142.0	196.3	24.7	28.7	41.4	12.9	16.0	20.4	2.6

Figure 1 shows EMS ± SE values for the discrepancies between the actual and calculated proportions of species in mixtures M1-M4. Alc showed the lowest discrepancies (0.017, 0.009 and 0.057 in M1, M2 and M4, respectively) compared to Alk or Ac whereas, in M3 the discrepancy was slightly higher (0.037) than that of Ac (0.027) which gave the lowest discrepancy in this mixture and the highest in all other mixtures. Lower discrepancies were obtained when markers were combined.

**Conclusion** For this particular set of mixtures, Alc had great potential to estimate composition of complex diets. However, the estimation from Ac was less good and Alk was intermediate. Estimation from the combination of these marker classes was always better than the poorest individual marker.

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**Figure 1** Mean square of errors (EMS±SE) between actual and calculated diet proportions for mixtures 1-4 calculated for different marker combinations

## Prediction of *in vivo* organic matter apparent digestibility of grass silage by means of the gas production technique

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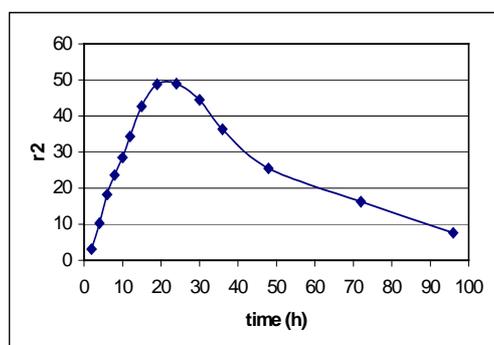
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**Introduction** In the Basque country and Galicia conditions, forage is usually conserved as silage, which accounts for a great part of cows winter rations. Robust techniques that predict the quality of forages for developing improved feeding strategies and screening of large number of forage samples are needed. In this sense, the gas production technique has proved to be of great interest when considering hays (Khazaal et al., 1995) and grass (Chenost et al., 2001), but information concerning grass silage *in vivo* data and gas production parameters is scarce. Therefore, the objective of this work is to try to assess *in vivo* grass silage apparent digestibility by means of gas production parameters.

**Materials and methods** Twenty-four grass silages of known *in vivo* organic matter apparent digestibility (OMD) were chosen from the sample bank collection of the CIAM on the basis of the range of variation of their OMD, from 508.6 to 804.8 g kg<sup>-1</sup>. The rumen liquor was obtained from four fistulated sheep receiving alfalfa hay and barley (70:30 DM), offered twice daily at 8:30 and 17:30 so as to satisfy 1.2 their maintenance requirements. Digesta samples were taken just before feeding (8:00), strained through 3 layers of cheesecloth, placed in a warmed thermos flask and transported to the laboratory. The composite inoculum of the four sheep was measured for pH and absorbance at 600nm using a 1:50 dilution. Preparation of buffer solutions was done the day before inoculation and liquor:medium (1:4) was as described by Mauricio et al. (1999). 500 mg DM of each sample was weighed into 125 ml serum bottles and were incubated in triplicate in a water bath kept at 39 ± 0.1°C. In vitro gas production was estimated using the Reading Pressure Technique. Head space pressure readings were taken at 2, 4, 6, 8, 10, 12, 15, 19, 24, 30, 36, 48, 72, and 96h post-inoculation with the accumulated gas released following each reading. Bottles were shaken just before each reading. Gas volumes were corrected for the gas released from controls and the quantity of OM incubated. Gas production profiles were determined in two different series that took place in different weeks. Accumulated gas production profiles were fitted to the following equation,  $p = A + B \cdot (1 - \exp(-c \cdot t))$  (Orskov and McDonald, 1979) where p is gas (ml) per incubated OM (g). At the end of each run pH was determined for each bottle.

**Results** The pH measurements (not reported) revealed that fermentations in bottles were satisfactory. Across silages, the correlation between accumulated gas production and OMD was highest at 24 h of incubation (figure 1). When considering the estimated parameters (table 1), the *in vivo* OMD values are better predicted from kinetic parameters, either gas production at 24h or c and from its combination with A and B but not by A+B which did not achieve significant relevance. The prediction accuracy was improved by adding the CP or NDF content of silage.



**Figure 1** Prediction precision of the OMD from accumulated gas production at a given time

**Table 1** OMD prediction equations from gas production parameters( A, B, c), accumulated gas production at 24h, CP or NDF

predicted equations*	R <sup>2</sup>	rsd
OMD=145.10-2.50(A)+0.99(B)+4990.12(c)	78.9	41.48
OMD=450.92+3.02(G24)-5.14(A)-1.35(B)	79.8	39.48
OMD=235.73+0.84(CP)+1.70(G24)-2.19(A)	81.3	37.95
OMD=568.05+1.62(G24)-3.51(A)-0.40(NDF)	82.2	37.04

\*: All included variables were significant, p<0.05

G24: Accumulated gas volume at 24h post inoculation

A, B, c: Estimated parameters (Orskov and McDonald, 1979)

CP: Crude Protein content, as g Kg<sup>-1</sup>

NDF: Neutro Detergent Fibre content, as g Kg<sup>-1</sup>

**Conclusions** According with the results, the gas test-technique alone or in combination with chemical analysis could be used to obtain a good estimation of the organic matter apparent digestibility of grass silage, similar to those found in other green or conserved forages.

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# The prediction of *in vivo* methane production and animal performance from the *in vitro* gas production technique

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**Introduction** The *in vivo* determination of methane (CH<sub>4</sub>) production requires specialist equipment which is costly to maintain. Whilst the *in vitro* gas production technique has been demonstrated to show potential to rank diets for their methanogenic potential at maintenance planes of nutrition (Moss and Givens, 1997) no study has investigated this relationship when feedstuffs are fed *ad libitum*. The objective of this study was to assess the ability of the technique to predict *in vivo* CH<sub>4</sub> production and animal performance from six diets differing in their chemical composition.

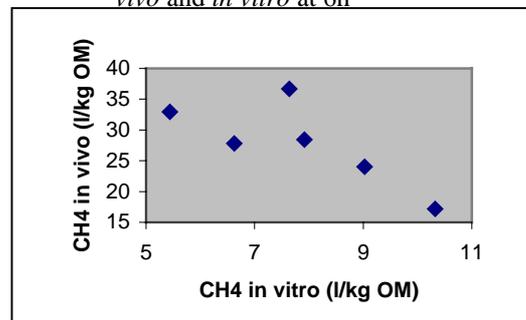
**Materials and Methods** Thirty-six Charolais cross heifers were fed one of six dietary treatments in a factorial arrangement over 75 days. The dietary treatments consisted of three grass silage to barley concentrate ratios (0.63:0.37, 0.40:0.60 and 0.10:0.90) with or without 350g of supplementary refined coconut oil. Animal performance in terms of CH<sub>4</sub> output using the SF<sub>6</sub> tracer gas technique and organic matter intake (OMI) were recorded. Each of these diets were incubated *in vitro* (1g) in four replicate incubations for 72 hours using a modification of the Mauchario *et al.* (1999) gas production technique. Fermentation gas CH<sub>4</sub> concentration was determined by gas chromatography. The model of France *et al.* (1993) was used to fit the cumulative gas and CH<sub>4</sub> production profiles. Stepwise regression analysis (MINITAB version 10) related cumulative gas and CH<sub>4</sub> production at any one time and modelled fermentation kinetics of total gas and CH<sub>4</sub> to the *in vivo* OMI and CH<sub>4</sub>/kgOMI.

**Results** Stepwise regression analysis revealed significant relationships between *in vitro* parameters and *in vivo* OMI and CH<sub>4</sub>/kgOMI (Table 1). Using the kinetics of fermentation to predict OMI and CH<sub>4</sub>/kgOMI resulted in more accurate predictions than using cumulative gas or CH<sub>4</sub> production at any given time. Multiple steps only improved the prediction of OMI when using the kinetics of total gas production. Single cumulative total gas and CH<sub>4</sub> production failed to provide significant (P > 0.05) predictive equations for *in vivo* CH<sub>4</sub>/kgOMI. In all instances the relationship between *in vivo* and cumulative *in vitro* CH<sub>4</sub>/kgOMI was negative (Figure 1). Methane production *in vitro* showed less variation than that observed *in vivo*.

**Table 1** Stepwise regression analysis relating *in vitro* gas production parameters to *in vivo* animal parameters.

Parameters \ Step	1	2	3	P	R <sup>2</sup> (adj)
<b>OMI</b>					
Cumulative total gas	Gas at 72h	-	-	0.038	62.6
Cumulative CH <sub>4</sub>	CH <sub>4</sub> at 72h	-	-	0.036	63.2
Total gas kinetics	Asymptote	Lag	μ6h	0.005	99.8
CH <sub>4</sub> kinetics	Asymptote	-	-	0.025	68.9
<b><i>In vivo</i> CH<sub>4</sub>/kgOMI</b>					
Total gas kinetics	μ8h	-	-	0.014	81.6
CH <sub>4</sub> kinetics	μ8h	-	-	0.009	85.2

**Figure 1** Relationship between l CH<sub>4</sub>/kgOM *in vivo* and *in vitro* at 6h



**Conclusion** The *in vitro* gas production technique demonstrated an ability to accurately predict the OMI and *in vivo* CH<sub>4</sub>/kgOMI as measured in beef animals, as well as successively identifying the inhibitory effect of coconut oil supplementation on CH<sub>4</sub> production regardless of the F:C ratio. Methane production increases with increased ruminal fermentation; this explains why *in vitro* CH<sub>4</sub>/kgOM increased linearly as the F:C ratio decreased. However under *ad libitum* feeding conditions post ruminal digestion of a diet also occurs and this explains the quadratic response to a decreasing F:C ratio. This work highlights the potential inadequacy of the *in vitro* system to assess diets for their methanogenic potential when rations differ in their chemical composition, but it does highlight the techniques potential to assess dietary treatments/additives which could serve as CH<sub>4</sub> inhibitors.

**Acknowledgements** The authors thank the support of the Environmental RTDI Programme 2000-2006, financed by the Irish Government under the National Development Plan and administered on behalf of the Department of the Environment and Local Government by the Environmental Protection Agency.

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## Seasonal variation in *in vitro* methane production of two perennial ryegrass cultivars

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**Introduction** Previous studies have identified small but significant differences in *in vitro* methane (CH<sub>4</sub>) production between perennial ryegrass cultivars harvested pre-heading date. This raises the possibility that enteric CH<sub>4</sub> production could be reduced through cultivar selection. The aim of this study was to assess the effect of harvest date on *in vitro* CH<sub>4</sub> output of two cultivars previously identified as having a high and low methanogenic potential (Lovett *et al.*, 2003).

**Materials and Methods** The two varieties used in this investigation were sourced from a larger trial comprising 18 varieties within a randomised block design with three replicates. Trial plots were harvested six times between 01/05/01 and 21/11/01, dried at 40°C for 48h and passed through a 1mm screen. Fermentability for each sample was determined singly *in vitro* (Mauricio *et al.* 1999) with rumen fluid derived from steers grazing permanent pasture. Fermentation gas CH<sub>4</sub> content was determined by gas chromatography. Extent of sample disappearance (g/kg) (DOMD) was calculated by weight difference for incubations of 8 and 72h. Methane gas fermentation kinetics were fitted to the model of France *et al.* (1993). Cultivar and harvest date effects were determined by ANOVA (Genstat Ed 6).

**Results** Harvest date significantly ( $P < 0.05$ ) modified all reported parameters except CH<sub>4</sub> production at 8h, while cultivar effects were significant ( $P < 0.05$ ) for DOMD at 8h, DOMD/CH<sub>4</sub> at 8h and the fractional rate of fermentation ( $\mu$ ) at time of half (T ½) the asymptote (A) (Table 1). More substrate is fermented per unit of CH<sub>4</sub> output for shorter than for longer incubations (8h vs. 72h). This reflects changes in the substrate being fermented over time for water soluble carbohydrates (WSC) whilst being more rapidly fermented than structural carbohydrates (SC) are also less methanogenic (Moe and Tyrrell, 1979). These differences in carbohydrate methanogenic potential also account for the declining DOMD/CH<sub>4</sub> relationship with harvest date for immature herbage is characterised as having high WSC levels and low SC while in mature herbage the reverse is true (Beever *et al.*, 1978).

**Table 1** Effect of variety and maturity on DOMD, CH<sub>4</sub> production and CH<sub>4</sub> fermentation kinetics

	DOMD (g/kg)		CH <sub>4</sub> (ml)		DOMD/CH <sub>4</sub>		Model Parameters		
	8h	72h	8h	72h	8h	72h	A ml	T ½ h	$\mu$ T ½
Cultivar Kells	312	697	8.64	39.65	37.2	17.6	42.05	19.47	0.0414
Cultivar Yatsyn 1	286	710	9.58	40.49	29.5	17.7	42.55	18.00	0.0453
s.e.d.	10	10	0.518	0.565	2.5	0.3	0.64	0.65	0.0012
Harvest 1	440	744	11.12	39.18	41.6	19.2	41.39	16.18	0.0441
Harvest 2	355	759	8.87	41.07	41.1	18.5	43.02	18.84	0.0448
Harvest 3	271	730	8.04	40.16	34.2	18.2	42.62	20.43	0.0398
Harvest 4	270	683	8.94	40.59	30.8	16.8	43.82	20.19	0.0386
Harvest 5	255	685	9.97	40.56	27.4	16.8	42.73	17.87	0.0436
Harvest 6	204	618	7.73	38.84	24.9	16.2	39.90	18.90	0.0490
s.e.d.	16	18	0.897	0.979	4.4	0.5	1.11	1.13	0.0022
P-cultivar	0.012	0.204	0.085	0.151	0.007	0.812	0.547	0.035	0.005
P-harvest	0.001	0.001	0.012	0.203	0.005	0.001	0.027	0.012	0.001
Interaction	0.054	0.952	0.044	0.022	0.514	0.121	0.008	0.093	0.269

**Conclusion** From these results although cultivar has no effect on the total volume of CH<sub>4</sub> produced, it can significantly reduce CH<sub>4</sub> production per unit of substrate disappearance (particularly for short ruminal incubations) and that this effect persists throughout the growing season.

**Acknowledgements** The authors thank the support of the Environmental RTDI Programme 2000-2006, financed by the Irish Government under the National Development Plan and administered on behalf of the Department of the Environment and Local Government by the Environmental Protection Agency.

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## Stable carbon isotope analysis of faecal and blood samples of sheep in relation to the diet

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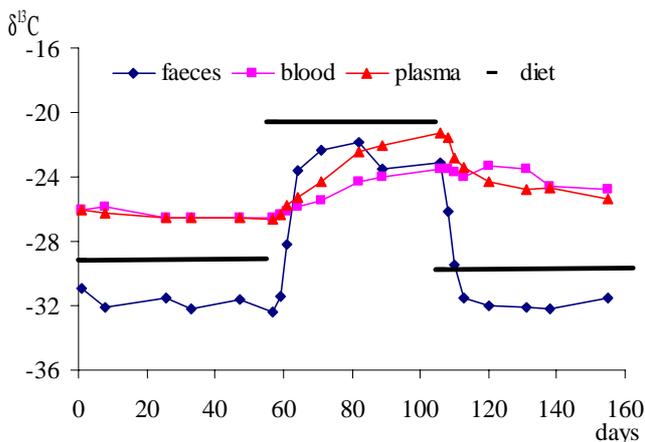
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**Introduction** Stable isotopes have been extraordinarily helpful in understanding animal migration, diet, food webs and nutrient flow (Hilderbrand *et al.*, 1996), based on the property that C<sub>3</sub> and C<sub>4</sub> plants possess distinctly different <sup>13</sup>C/<sup>12</sup>C ratios ( $\delta^{13}\text{C}$  value) due to isotopic fractionation during photosynthetic carbon fixation (Smith & Epstein, 1971). Most woody species and temperate graminoids assimilate carbon via the Calvin cycle (C<sub>3</sub>), which discriminates stronger against the heavier isotope (<sup>13</sup>C) than Hatch-Slack (C<sub>4</sub>) species (tropical and subtropical graminoids and some shrubs). C<sub>3</sub> and C<sub>4</sub> plant species have mean  $\delta^{13}\text{C}$  values of -27 ‰ and -13 ‰ respectively (O'Leary, 1981). DeNiro & Epstein (1978) were one of the first to show that the isotopic composition of the whole animal body is similar to that of its diet. Other authors have also found relationships between the isotopic composition of animal tissues and the diet (González-Martin *et al.*, 1999; Jones *et al.*, 1979). The aim of this study was to investigate stable carbon isotope composition in sheep fed diets consisting of either C<sub>3</sub> or C<sub>3</sub>+C<sub>4</sub> plants.

**Materials and methods** Four rumen cannulated mature cross-bred wethers (average live weight 77.1 (sd 10.4) kg at the start of the trial) were housed in individual pens. The animals were fed a C<sub>3</sub>-plant diet in the first experimental period (56 days), a combination of C<sub>3</sub> and C<sub>4</sub> plants in the second period (49 days), and again a C<sub>3</sub>-plant diet in the third period (49 days). Water was available *ad libitum*. The C<sub>3</sub> plant was ryegrass hay and the C<sub>4</sub> plant was maize silage. The ratio hay/maize silage was 54/46 (on DM basis) in the second period. Before the start of the experiment the sheep were held on pasture (period 0). At regular times, blood and faecal grab samples were taken. Jugular vein blood samples were collected in EDTA-tubes, partly frozen immediately at -18°C for analysis of whole blood and partly centrifuged (4000 g, 10 minutes, 4°C) for analysis of plasma after storage at -18°C. Faecal samples were dried at 100°C for 24 hours before grinding in a planetary ball mill (< 1 µm) (PM 400, Retch, Germany). The samples were transferred into tin capsules for isotope analysis in duplicate with an elemental analyser (ANCA-SL, EUROPA PDZ, UK), connected to a Continuous Flow Isotope Ratio Mass Spectrometer (model 20-20, EUROPA PDZ, UK). The  $\delta^{13}\text{C}$  value (expressed in parts per thousand, ‰) is a relative measurement of the <sup>13</sup>C/<sup>12</sup>C ratio, made against a laboratory (working) reference, which is in turn calibrated against an international standard. Mean values were compared using ANOVA and t-tests.

**Results** The  $\delta^{13}\text{C}$  value for the feeds were: pasture period 0, -30.4 ‰; hay period 1, 2 and 3 respectively -29.2 ‰, -28.9 ‰, -29.8 ‰; maize silage period 2, -10.8 ‰. The  $\delta^{13}\text{C}$  value of the hay/maize silage diet in period 2 was -20.6 ‰. The evolution of the mean  $\delta^{13}\text{C}$  values of the animal samples is given in Figure 1. Animal variability was low (mean CV 1.2, 1.8 and 2.6 % for plasma, blood and faeces respectively). At all time points except for the first five days in period 3, the  $\delta^{13}\text{C}$  value of faeces was lower than the dietary  $\delta^{13}\text{C}$  value (P<0.000). On the other hand, the values for blood and plasma were higher than the dietary  $\delta^{13}\text{C}$  value in period 1 and 3 (P<0.000), and lower in period 2 (P<0.000) at all time points. Figure 1 also shows the large differences between the  $\delta^{13}\text{C}$  values for faeces compared to whole blood or plasma in period 1 and 3, but not in period 2, suggesting that sample type differences are depending also on the diet. Following the dietary change in period 2, the change in  $\delta^{13}\text{C}$  value was slower in blood and plasma than in faeces. The  $\delta^{13}\text{C}$  values in period 2 were different (P<0.05) from the mean values in period 1 from day 5 (faeces, plasma) and day 8 (whole blood) after the dietary change.



**Figure 1** Mean  $\delta^{13}\text{C}$  value (‰) of faeces, blood, plasma and diet

**Conclusion** Stable carbon isotope analysis of faecal, blood and plasma samples has potential to discriminate between a C<sub>3</sub> and a mixed C<sub>3</sub>/C<sub>4</sub> diet. However, differences between different types of samples for the mean  $\delta^{13}\text{C}$  value and for the rate of change following a dietary change need further investigation.

**Acknowledgements** This work was supported by the Ministry of Small Enterprises and Agriculture, Directorate Research and Development, Brussels.

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## Microbial protein kinetics for sheep fed with three different hays using <sup>15</sup>N as marker

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**Introduction.** Non-degradable dietetic protein supply depends on rumen degradation. Determination of microbial protein synthesized in the rumen as the result of microbial fermentation is important because microbial protein synthesis could be influenced by diet (Dove and Milne, 1994). Several evaluation systems consider the contribution of microbial protein on protein intestinal flow as a constant, based on feed intake. But this approach has presented great variations. The purpose of this work was to determine *in vivo* microbial protein kinetics for sheep fed with three protein level hays.

**Material and methods.** Three sun-dried hays were chosen according to their crude protein (CP) content: LUC – Lucerne (*Medicago sativa*), SIG – signalgrass (*Brachiaria decumbens*) and TIF – Tifton-85 (*Cynodon sp.*). The animals used were Santa Inês wether (LW = 40 ± 5.7 kg) with permanent rumen and duodenum cannulas. 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 (diets), 3 periods and 6 animals. As microbial marker, 1 mg of <sup>15</sup>N from the salt (<sup>15</sup>NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> with isotopic enrichment of 90% was applied per kg of body weight. The marker was applied in the morning immediately before the first meal in a pulse dose. Collections of duodenum digesta were taken at 0, 4, 8, 12, 16, 20, 24, 26, 30, 34, 38, 42 and 46 h after marker introduction. Determination of <sup>15</sup>N in the microbial biomass presented in duodenum digesta was made by mass spectrometry. Data were fitted by Grovum and Williams (1973) model. Means were compared by standard error of difference between means (sed) and Student t test at probability level of 5% (SAS, 2000).

**Results.** Chemical composition of tested feeds is illustrated on Table 1. LUC presented the highest level for CP and SIG, the lowest. According to Van Soest (1994), the minimum level of CP of diets for ruminants intending the N supply to not compromise rumen microbial activity should be 60 - 80 g.kg<sup>-1</sup> DM. Thus, in this experiment, treatments were disposed above (LUC), under (SIG) and in (TIF) this limit. Regarding the microbial protein transit, main differences were noticed between treatments SIG or TIF and treatment LUC (Table 2). SIG and TIF promoted microbial growth kinetics and microbial protein transit from rumen to duodenum in a similar way. Nitrogen assimilation by rumen microorganisms (k<sub>1</sub>) was much faster (P < 0.01) for LUC, showing that LUC nitrogen was more available than the other two treatments. Microbial protein produced from LUC also had a faster passage (k<sub>2</sub>) from rumen to duodenum. The other parameters also illustrated a better use of LUC nitrogen.

**Table 1** Chemical composition (g.kg<sup>-1</sup>DM) of Lucerne (LUC), signalgrass (SIG) or Tifton-85 (TIF) hays

components <sup>†</sup>	treatments			sed <sup>‡</sup>
	LUC	SIG	TIF	
DM <sup>¶</sup>	841.7 <sup>c</sup>	851.9 <sup>a</sup>	848.5 <sup>b</sup>	1.25
OM	900.3 <sup>c</sup>	925.5 <sup>a</sup>	907.1 <sup>b</sup>	2.92
NDF	520.8 <sup>c</sup>	777.9 <sup>b</sup>	803.5 <sup>a</sup>	11.02
ADF	417.7 <sup>b</sup>	470.2 <sup>a</sup>	460.7 <sup>a</sup>	8.32
ADL	105.7 <sup>a</sup>	60.7 <sup>b</sup>	65.6 <sup>b</sup>	3.96
CP	190.8 <sup>a</sup>	29.0 <sup>c</sup>	75.1 <sup>b</sup>	2.89

<sup>†</sup> DM: dry matter; OM: organic matter; NDF: neutral detergent fibre; ADF: acid detergent fibre; ADL: acid detergent lignin; CP: crude protein

<sup>‡</sup> standard error of difference between means

<sup>¶</sup> g.kg<sup>-1</sup> fresh matter

<sup>a, b, c</sup> means followed by different superscripts, in row, are significantly different (P < 0.05)

**Table 2** Microbial protein transit kinetics parameters for sheep fed with Lucerne (LUC), signalgrass (SIG) or Tifton-85 (TIF) hays

parameters <sup>†</sup>	treatments			sed <sup>‡</sup>
	LUC	SIG	TIF	
k <sub>1</sub> (h <sup>-1</sup> )	0.048 <sup>a</sup>	0.027 <sup>b</sup>	0.027 <sup>b</sup>	0.0053
k <sub>2</sub> (h <sup>-1</sup> )	0.518 <sup>a</sup>	0.280 <sup>b</sup>	0.354 <sup>b</sup>	0.0419
TT (h)	9.84 <sup>b</sup>	16.11 <sup>a</sup>	14.11 <sup>a</sup>	1.775
MRT <sub>pool1</sub> (h)	21.87 <sup>b</sup>	39.73 <sup>a</sup>	44.06 <sup>a</sup>	4.041
MRT <sub>pool2</sub> (h)	1.94 <sup>b</sup>	3.74 <sup>a</sup>	3.24 <sup>a</sup>	0.444
TMRT (h)	33.65 <sup>b</sup>	59.58 <sup>a</sup>	61.41 <sup>a</sup>	3.333

<sup>†</sup> k<sub>1</sub>: nitrogen incorporation rate; k<sub>2</sub>: passage rate; TT: transit time; MRT<sub>pool1</sub> and MRT<sub>pool2</sub>: mean retention times in pools 1 and 2; TMRT: total mean retention time

<sup>‡</sup> standard error of difference between means

<sup>a, b, c</sup> means followed by different superscripts, in row, are significantly different (P < 0.05)

**Conclusion** The protein level of diets is directly related to microbial synthesis and microbial N supply for ruminants. Lucerne hay promoted the fastest nitrogen incorporation by rumen microorganisms and that produced protein became available in a shorter time than Tifton-85 or signalgrass hays.

**Acknowledgements** This experiment is part of a project supported by FAPESP.

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# Voluntary feed intake, apparent digestibilities and nutritive values in ponies given *ad libitum* access to complete pelleted diets made from wheat straw and unmolassed sugar beet pulp

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**Introduction** Although overall intake figures were high, critical levels (~400 g/kg DM) of unmolassed sugar beet pulp (USBP) inclusion suppressed dry matter intake (DMI) in ponies by up to 35% when included in complete pelleted diets made with dried grass (Hyslop, 2002). Dulphy *et al* (1997) have concluded that horses consume straw-based forages at lower levels than grass or legume-based forages. Given this observation with straw based diets, the objective of this study was to examine DMI, *in vivo* apparent digestibilities and nutritive values in ponies offered pelleted complete diets made from ground wheat straw and containing USBP at inclusion levels between 400 – 800 g/kg DM.

**Materials and methods** 6 mature Welsh-cross pony geldings (mean LW 275 kg) were individually housed and offered one of 6 diets *ad libitum*. Along with 1 kg/head/day of grass hay (HAY), five of the diets offered *ad libitum* were complete, pelleted diets (CD4, CD5, CD6, CD7 and CD8) formulated from varying amounts of ground wheat straw (WS) and USBP such that USBP inclusion levels were 400, 500, 600, 700 and 800 g/kg DM respectively. Other CD constituents were soyabean meal (75 g/kg DM), minerals (25 g/kg DM) and molasses (25 g/kg DM) with the remainder being WS. The 6<sup>th</sup> diet on offer was *ad libitum* HAY. Feed composition in diets CD4, CD5, CD6, CD7, CD8 and HAY were as follows:- DM 868, 848, 839, 834, 832, 816 (g/kg); crude protein (CP) 115, 104, 98, 107, 112, 65 (g/kg DM); and neutral detergent fibre (NDF) 430, 515, 514, 512, 484, 794 (g/kg DM). The experiment was a 6 x 4 partially balanced incomplete block changeover design lasting for 4 periods of 21 days. Each 21 day period consisted of a 16 day adaptation and a 5 day recording phase when 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 analysed using the residual maximum likelihood (REML) facility in Genstat 5. *In vivo* apparent digestibilities, DE and DCP contents for each CD alone were estimated by difference from total diet values and for USBP alone by linear regression.

**Results** Voluntary DMI of CD's declined on average by 47% from 9.19 to 4.86 kg/d as USBP inclusion increased (Table 1). Despite this decline, DMI of HAY offered *ad libitum* at 4.59 kg/d was lower than total DMI (TDMI) when all but CD7 and CD8 were offered. For both the total diets (Table 1) and the CD's calculated by difference (Table 2), *in vivo* apparent fibre digestibilities and DE contents generally increased as USBP inclusion level increased whilst generally, CPD declined. HAY digestibilities and nutritive values were low. Estimated DE content for USBP alone was 13.5 MJ/kg DM but estimated DCP content at 39 g/kg DM was low reflecting the low estimated CPD of 383 g/kg.

**Table 1.** DMI, *in vivo* apparent digestibilities, DE (MJ/kg DM) and DCP (g/kg DM) contents of total diets offered.

	CD4	CD5	CD6	CD7	CD8	HAY	sed		CD4	CD5	CD6	CD7	CD8	HAY	sed
CD (kg/d)	9.19 <sup>a</sup>	9.27 <sup>a</sup>	7.40 <sup>a</sup>	5.20 <sup>b</sup>	4.86 <sup>b</sup>	0 <sup>c</sup>	0.717	(g/kg)							
HAY “	0.60 <sup>a</sup>	0.42 <sup>a</sup>	0.29 <sup>a</sup>	0.51 <sup>a</sup>	0.62 <sup>a</sup>	4.59 <sup>b</sup>	0.357	DMD	541 <sup>a</sup>	558 <sup>a</sup>	602 <sup>b</sup>	646 <sup>c</sup>	686 <sup>c</sup>	408 <sup>d</sup>	18.9
TDMI “	9.71 <sup>a</sup>	9.71 <sup>a</sup>	7.74 <sup>b</sup>	5.64 <sup>c</sup>	5.50 <sup>c</sup>	4.59 <sup>c</sup>	0.789	OMD	569 <sup>a</sup>	581 <sup>a</sup>	624 <sup>b</sup>	675 <sup>c</sup>	715 <sup>d</sup>	421 <sup>e</sup>	17.3
g/kg LW	35.6 <sup>a</sup>	33.4 <sup>a</sup>	27.9 <sup>b</sup>	21.8 <sup>c</sup>	19.8 <sup>c</sup>	17.2 <sup>c</sup>	0.210	CPD	598 <sup>a</sup>	559 <sup>a</sup>	451 <sup>ab</sup>	463 <sup>ab</sup>	484 <sup>ab</sup>	336 <sup>b</sup>	67.2
g/kgLW <sup>0.75</sup>	145 <sup>a</sup>	138 <sup>a</sup>	114 <sup>b</sup>	87 <sup>c</sup>	81 <sup>c</sup>	70 <sup>c</sup>	9.18	ADFD	228 <sup>a</sup>	293 <sup>ab</sup>	336 <sup>b</sup>	440 <sup>c</sup>	532 <sup>c</sup>	318 <sup>ab</sup>	42.5
DE	10.5 <sup>a</sup>	10.5 <sup>a</sup>	11.1 <sup>a</sup>	11.1 <sup>a</sup>	12.2 <sup>b</sup>	6.8 <sup>c</sup>	0.41	NDFD	329 <sup>a</sup>	429 <sup>b</sup>	514 <sup>c</sup>	593 <sup>d</sup>	628 <sup>d</sup>	412 <sup>b</sup>	28.7
DCP	66 <sup>a</sup>	65 <sup>ab</sup>	45 <sup>cd</sup>	46 <sup>bc</sup>	52 <sup>abc</sup>	25 <sup>d</sup>	8.84	GED	570 <sup>a</sup>	570 <sup>a</sup>	609 <sup>ab</sup>	628 <sup>b</sup>	678 <sup>c</sup>	376 <sup>d</sup>	20.0

For both tables, values not sharing common superscripts differ significantly (P<0.05) except HAY where (P<0.001).

**Table 2.** *In vivo* apparent digestibilities (g/kg), DE (MJ/kg DM) and DCP (g/kg DM) contents of each CD and HAY.

	CD4	CD5	CD6	CD7	CD8	HAY	sed		CD4	CD5	CD6	CD7	CD8	HAY	sed
DMD	551 <sup>a</sup>	563 <sup>a</sup>	609 <sup>b</sup>	674 <sup>c</sup>	718 <sup>d</sup>	408 <sup>e</sup>	19.1	NDFD	316 <sup>a</sup>	431 <sup>b</sup>	518 <sup>c</sup>	626 <sup>d</sup>	671 <sup>d</sup>	411 <sup>b</sup>	29.6
OMD	574 <sup>a</sup>	586 <sup>a</sup>	632 <sup>b</sup>	709 <sup>c</sup>	750 <sup>d</sup>	420 <sup>e</sup>	18.2	GED	582 <sup>a</sup>	578 <sup>a</sup>	617 <sup>ab</sup>	659 <sup>b</sup>	714 <sup>c</sup>	375 <sup>d</sup>	21.6
CPD	603 <sup>a</sup>	610 <sup>a</sup>	459 <sup>ab</sup>	467 <sup>ab</sup>	495 <sup>ab</sup>	342 <sup>b</sup>	79.9	DE	10.7 <sup>a</sup>	10.7 <sup>a</sup>	11.3 <sup>a</sup>	11.6 <sup>a</sup>	12.8 <sup>b</sup>	6.8 <sup>c</sup>	0.44
ADFD	220 <sup>a</sup>	285 <sup>ab</sup>	336 <sup>b</sup>	475 <sup>c</sup>	569 <sup>c</sup>	319 <sup>ab</sup>	45.6	DCP	69 <sup>a</sup>	67 <sup>a</sup>	46 <sup>b</sup>	49 <sup>ab</sup>	55 <sup>ab</sup>	25 <sup>c</sup>	9.24

**Conclusions** High DMI's were observed in ponies offered ground and pelleted complete diets even when low quality wheat straw was the basal ingredient and USBP inclusion levels were high. However, this study confirms that increasing the USBP content of equine diets progressively reduces voluntary DMI and increases the DE content.

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

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## ***In vitro* methane production of different cultivars of perennial ryegrass**

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**Introduction** Irish livestock production systems are characterised by the high utilization of grazed grass combined with minimal supplementary feeding. As such the options to reduce methane (CH<sub>4</sub>) production, particularly for beef animals, is limited to the finishing period when diet can be controlled and varied. No data is available regarding the methanogenic potential of differing grass cultivars. This study assessed CH<sub>4</sub> output, as measured *in vitro*, of six ryegrass cultivars, with the aim of quantifying the potential for enteric CH<sub>4</sub> emissions to be reduced from ruminants grazing ryegrass dominant swards.

**Materials and Methods** 18 diploid perennial ryegrass varieties were established in 1999 in a randomised block design with three replicates at the Crop Testing Centre Backwestern. Six varieties (consisting of two Early, two Intermediate and two Late maturity classes) were selected in 2001. Plots were harvested on six occasions (between the 01/05/01 and 21/11/01) when grass cover achieved 2000 kg Dry Matter hectare (DM/ha). After harvesting the samples were oven dried (40°C for 48h) and subsequently milled through a 1mm screen. Only samples harvested on the 01/05/01 were used, with each sample (0.5g) being incubated for a total of 72h using a modified gas production procedure of Mauricio *et al.* (1999). Methane production was determined by analysis of the fermentation gases by gas chromatography. The extent of sample disappearance (DOMD) was determined by weight difference following oven drying and ashing of the filtrate residue 8, 24 and 72h post inoculation. Fermentation kinetics per gram of organic matter (OM) for CH<sub>4</sub> were determined using the model of France *et al.* (1993). Cultivar effects were identified using ANOVA (Genstat Edition 6).

**Results** The asymptote (A ml) of CH<sub>4</sub> production varied from 27.05 to 30.33 ml (Table 1) and was significantly modified (P = 0.005) by variety despite variety having no significant effect (P > 0.05) on digestibility. This suggests that the partitioning of energy derived from substrate fermentation is modified by variety. Comparisons between differing varieties revealed significant differences between all modelled fermentation parameters although the overall variety effect was only significant (P < 0.05) for the A and the fractional rate of fermentation ( $\mu$ ) at half the asymptote (T ½). The significance of variety on CH<sub>4</sub> production increased with an increase in the duration of incubation (P = 0.491, 0.037 and 0.003 at 8, 24 and 72h respectively).

**Table 1** Effect of variety on DOMD, CH<sub>4</sub> production and CH<sub>4</sub> fermentation kinetics (per g of OM)

Variety	DOMD g/kg			CH <sub>4</sub> ml			Model parameters				
	8h	24h	72h	8h	24h	72h	A ml	Lag h	$\mu$ 8h	T ½	$\mu$ T ½
Moy	427	646 <sup>a</sup>	737	9.32	21.44 <sup>ab</sup>	27.55 <sup>a</sup>	27.17 <sup>a</sup>	2.19 <sup>a</sup>	0.0749 <sup>c</sup>	11.44 <sup>a</sup>	0.0748 <sup>d</sup>
Yatsyn 1	439	667 <sup>ab</sup>	738	9.09	22.61 <sup>b</sup>	30.46 <sup>c</sup>	30.33 <sup>c</sup>	2.22 <sup>a</sup>	0.0656 <sup>ab</sup>	12.68 <sup>bc</sup>	0.0646 <sup>ab</sup>
Kells	437	694 <sup>b</sup>	758	8.64	20.39 <sup>a</sup>	27.54 <sup>a</sup>	27.60 <sup>a</sup>	2.23 <sup>ab</sup>	0.0646 <sup>a</sup>	12.82 <sup>c</sup>	0.0629 <sup>a</sup>
Respect	415	673 <sup>ab</sup>	719	8.96	21.52 <sup>ab</sup>	28.25 <sup>ab</sup>	27.83 <sup>ab</sup>	2.26 <sup>ab</sup>	0.0708 <sup>abc</sup>	12.09 <sup>abc</sup>	0.0711 <sup>bcd</sup>
Antara	412	684 <sup>b</sup>	731	9.70	22.49 <sup>b</sup>	29.41 <sup>bc</sup>	29.03 <sup>bc</sup>	2.22 <sup>a</sup>	0.0725 <sup>bc</sup>	11.74 <sup>ab</sup>	0.0715 <sup>cd</sup>
P'stewart	416	692 <sup>b</sup>	760	8.06	20.27 <sup>a</sup>	27.49 <sup>a</sup>	27.05 <sup>a</sup>	2.35 <sup>b</sup>	0.0666 <sup>ab</sup>	12.73 <sup>bc</sup>	0.0664 <sup>abc</sup>
s.e.d.	24	17	23	0.82	0.73	0.62	0.69	0.06	0.0033	0.53	0.0033
P-cultivar	0.790	0.060	0.309	0.491	0.037	0.003	0.005	0.171	0.051	0.114	0.003

**Conclusion** From this small sample size of six perennial ryegrass varieties it appears that CH<sub>4</sub> emissions arising from enteric fermentation could be manipulated by variety selection. However, it must be stressed that the overall significance of variety was lower for the shorter than for the extended incubation times and that consequently the overall effect of variety selection may well be less than the 0.12 difference observed in terms of the asymptote of CH<sub>4</sub> production. That differences between varieties in terms of CH<sub>4</sub> production per unit of fermented substrate exist suggests that plant breeders may have a potential role in developing grasses of a reduced methanogenic potential.

**Acknowledgements** The authors thank the support of the Environmental RTDI Programme 2000-2006, financed by the Irish Government under the National Development Plan and administered on behalf of the Department of the Environment and Local Government by the Environmental Protection Agency.

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# ***In vitro* methane production as influenced by harvest date and level of nitrogen application**

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**Introduction** Increasing animal productivity increases daily enteric methane (CH<sub>4</sub>) emissions but reduces CH<sub>4</sub> output per unit of animal production. Irish livestock production systems are characterised by a high dependence on grazed pasture. Increased nitrogen (N) fertiliser application can increase animal productivity through increased intake. The objective of this trial was to assess the effect of N level application and pasture maturity on *in vitro* methane production.

**Materials and Methods** 72 grass samples consisting of two *Lolium* perenne hybrids harvested as three 6-7 week regrowths (02/07/01-08/10/01) and three levels of N application (0-160 kgN/ha/growth) were obtained from an agronomy trial (split-plot design with four replicate blocks). Samples were dried (48 hours at 55°C) and milled through a 1mm screen. Each sample was incubated singly (0.15g) for 8 and 24 hours only using a modified gas production procedure of Mauricio *et al.* (1999) which facilitated the sampling of the fermentation gases and their subsequent analysis for CH<sub>4</sub> content by gas chromatography. The extent of sample disappearance (DOMD) was determined by weight difference following oven drying and ashing of the filtrate residue. The treatment effects of harvest date and level of N-application were analysed by ANOVA (Genstat Edition 6).

**Results** No significant hybrid effects were identified, thus only the main effects of harvest date and N application level are reported (Table 1). All measured parameters increased with increasing duration of incubation. Increased N application level significantly ( $P < 0.05$ ) reduced total gas production, DOMD and CH<sub>4</sub> production. Regression analysis revealed a highly significant ( $P < 0.001$ ) relationship between DOMD and total gas and CH<sub>4</sub> production.

**Table 1** Main treatment effects on the *in vitro* fermentation and degradation of 72 grass samples

Main effect Treatment	Harvest				N Level (kgN/ha/growth)				P-Harvest	P-N Level
	02/07/01	20/08/01	08/10/01	sed	0	80	160	sed		
Total gas 8h <sup>#</sup>	106	100	109	4	114	104	96	5	0.079	0.007
Total gas 24h <sup>#</sup>	219	215	226	8	237	217	206	7	0.223	0.008
CH <sub>4</sub> 8h <sup>#</sup>	5.56	5.74	6.25	0.31	6.44	6.04	5.07	0.23	0.089	0.001
CH <sub>4</sub> 24h <sup>#</sup>	17.91	18.20	19.38	0.68	19.91	18.53	17.04	0.90	0.089	0.025
DOMD 8h <sup>##</sup>	395	370	414	20	431	380	367	30	0.104	0.125
DOMD 24h <sup>##</sup>	688	683	712	15	742	684	657	19	0.146	0.002

<sup>#</sup> expressed as mL of gas per g OM, <sup>##</sup> expressed as g/kg DM

**Conclusion** The effect of harvest date on DOMD agrees with Givens *et al.* (1993), but the negative effect of N application level on DOMD is contrary to that identified by Delagaed *et al.* (1997). The decline in CH<sub>4</sub> production associated with increased N application level is primarily derived from reduced substrate fermentation as identified by regression analysis. Part of the reduction may also arise from an expected increase in pasture crude protein content, for different chemical components have varying degrees of association with CH<sub>4</sub> (Holter and Young, 1992). If the *in vitro* response in CH<sub>4</sub> production to increased N application were replicated *in vivo*, the *in vivo* response could be even greater as increased N application usually increases intake (Delagaed *et al.*, 1997). However this might not occur if the drop in DOMD due to higher N application was also replicated *in vivo*. In terms of total green house gas emissions from the agricultural sector, any reduction in CH<sub>4</sub> production would need to be balanced against increased N<sub>2</sub>O emissions.

**Acknowledgements** The authors thank the support of the Environmental RTDI Programme 2000-2006, financed by the Irish Government under the National Development Plan and administered on behalf of the Department of the Environment and Local Government by the Environmental Protection Agency. Samples were obtained from an EU Fifth Framework Programme funded project (Sweetgrass)

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# Digestible dry matter and protein of lucerne hay or lucerne silage treated with sulphuric acid using mobile nylon bag technique

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**Introduction** Legume forages, because of the tubular and hollow stem structure, low soluble sugar content and high buffering capacity are less likely to undergo optimal fermentation. Therefore, response to silage additives (such as formic acid) or preservatives may be great with legume forages. This study was conducted to evaluate the DM and protein digestibility of lucerne hay or lucerne silage treated with urea and/or sulphuric acid using mobile nylon bag technique.

**Materials and methods** Second growth lucerne (about 33% DM) was harvested and dried or chopped then ensiled with urea (0.0 and 0.5% of DM) and/or sulphuric acid (0.0 and 0.6% of DM) in a complete randomized design (LH: lucerne hay, LS: lucerne silage, LS+U: LS + 0.5% urea, LS+SA: LS + 0.6% acid, LS+U,SA: LS + 0.5% urea + 0.6% acid). The chemical composition of the silages were determined after 35 days of ensiling. The standard procedures were used to determine the chemical composition of the samples. PH and N- NH<sub>3</sub> were determined in the silage extraction. CP and NPN were determined in dry samples. The ruminal, post ruminal and total tract disappearances of dry matter and protein were determined using the mobile nylon bag technique (Mesgaran, 2002). Four Holstein steers (400±15 Kg) fitted with the rumen fistula and T- shaped cannulae were used in the present study. They fed 2.5 kg DM lucerne hay, 1.4 kg DM maize silage, 0.5 kg DM wheat straw and 2.4 kg DM concentrate (16.5 % CP) per steer per day. The bags (3x6 cm) were made of Dacron cloth with a pore size of 46 µm. About 1.2 g dry matter of the samples (grounded through 2 mm screen) was placed in each bag (24 bags per each treatment). Sixteen bags inserted into plastic mesh cylinders (26x8 cm, 0.57 mm pore size) and incubated in the rumen for 12 h. All bags were removed at once and washed in running cold water. Eight bags of each treatment after incubation in the rumen and eight bags containing intact samples were then inserted into small intestine via the cannulae at the rate of one bag every 30 min and removed from the voided faeces, washed in cold running water. Finally, the bags were dried in a forced air oven (58 °C, for 24 h), then weighed to determine dry matter disappearance. Data were analyzed using the GLM procedure of SAS. Statistical significance was determined at P<0.05.

**Results** Chemical composition of lucerne hay or the silages are shown in Table 1. The data related to the disappearance of DM and protein from mobile nylon bags within rumen, intestine and total tract are shown in Table 2. The addition of urea to the silage increased significantly PH and CP content (P<0.05). Using sulphuric acid as an additive for lucerne silage caused to reduce PH and N-NH<sub>3</sub> content of the silages (P<0.05). Ruminal, post ruminal and total tract disappearance of dry matter and protein influenced by the treatments (P<0.05).

**Table 1** Chemical composition of lucerne silage treated with urea and sulfuric acid

Items	LS	LS+U	LS+SA	LS+U,SA	SEM	P
PH	4.49	4.81	4.11	4.33	0.10	*
CP (g kg <sup>-1</sup> )	181	183.2	174.6	189.6	8.3	*
NPN((g kg <sup>-1</sup> )	19.0	13.6	13.0	15.45	2.2	*
N-NH <sub>3</sub> (mg dl <sup>-1</sup> )	12.2	13.0	10.7	10.12	1.7	*

\* P<0.05

**Table 2** The disappearance (g kg<sup>-1</sup>) of DM and protein of lucerne hay and lucerne silage treated with urea and sulphuric acid from mobile bags within rumen, intestine and total tract

Item	LH	LS	U+LS	SA+LS	U,SA+LS	SEM	P
Intact feed – disappearance in the rumen							
DM	465	560	594	552	529	28	*
Protein	735	857	820	742	667	34	*
Intact feed – disappearance in the intestine							
DM	558	573	590	620	628	37	*
Protein	861	885	892	913	915	8	*
Intact feed – disappearance in the total tract							
DM	575	573	620	645	666	7	*
Protein	901	916	936	952	956	21	*

\* P<0.05

**Conclusion** It has been indicated that the urea, as an additive for lucerne silage, increased CP of the silages. While the NPN content of the silages treated with urea was lower compared with LS. Sulphuric acid can also be used as an additive in lucerne silage as it caused to increase the DM and protein digestibility of the silages. So, it has concluded that both urea and sulphuric acid may be used as a good preservative in lucerne silage.

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# Effect of urea-whey treatment on the chemical composition and digestibility of wheat straw.

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**Introduction** Recently, in Iran, increasing surplus of whey, a by-product of cheese producing plants are becoming an environmental problem and this is due to dairy development programs. Whey dumped into the plant disposal systems insert a high pollution load to the environment. Whey is a valuable source of carbohydrates, a good supply of energy and contains high quality protein and minerals. Options such as condensation and/ or fractionation also dehydration are too costly, and requires high technology. Finding an alternative method for preserving and using of whey could reduce the environmental risks, cost and associated difficulties. The use of liquid whey has been used for many years as a supplemental feed for ruminants. Like molasses, liquid whey can be a carrier of non-protein nitrogen components such as urea or ammonium salts to make crude protein supplements (Khattab *et al.* 2000). Therefore, the objective of the present study was to find a simple method to preserve and improve the utilisation of fresh whey in animal nutrition as well as a desirable way to reduce environmental pollution.

**Materials and Methods** A 5×4 factorial completely randomized design including five treatments and four periods of time was conducted in which, wheat straw was treated with urea, water, whey and sodium chloride and sealed in plastic bags (each with four replicates). The basic ratios among straw, water, urea and salt were 100:75:4.5:1.5, respectively (Treatment I), but water was substituted with whey in amounts of 25, 50, 75 and 100% in other treatments. After 20, 40, 60 and 80-day periods, respectively the bags were opened and sampled for measurement of pH, chemical analysis and *in vitro* digestibility studies. Data were analysed for parametric statistics, using a fixed factorial model of analysis of variance (ANOVA).

**Results** The effects of treatments are presented in Table 1. Using of whey instead of water, increased the pH values of the urea treated straw, but significant (P<0.05) difference was observed only at 25% of substitution. By inclusion of whey, the amount of Ash was increased in the treated straw, however it became statistically significant (P<0.05) when water was completely substituted with whey. Crude fibre (CF) content of treated straw was significantly (P<0.05) decreased when 75 or 100% of water was substituted with whey. Digestibility of dry mater (DMD) and organic mater (OMD) were significantly (P<0.05) higher in all treatments, which contained whey, however the highest amount obtained at 100% whey. Crude protein (CP), gross energy (GE) and nitrogen free extract (NFE) were not changed among the treatments. Regarding to the time periods, no significant differences were observed in pH, Ash, EE, NFE and GE when treatments ensiled for 20, 40, 60 or 80 days. Only CP content was significantly (P<0.05) increased after 40 days (Table 2). The CF content of all treatments was negatively affected (P<0.05) by period of ensiling whereas, the digestibilities of DM and OM were increased after 40 days. There were no any significant interactions between treatments and ensiling periods for the above parameters.

Table 1. Effect of treatments on the chemical composition, digestibility (g/100g DM) and GE (cal/g DM) of wheat straw.

Item	Treatments					s.e	sol
	I	II	III	IV	V		
PH	7.97 <sup>b</sup>	8.24 <sup>a</sup>	8.13 <sup>ab</sup>	8.03 <sup>ab</sup>	8.1 <sup>b</sup>	.11	*
Ash	11.9 <sup>b</sup>	12.2 <sup>ab</sup>	12.6 <sup>ab</sup>	13.5 <sup>ab</sup>	14.2 <sup>a</sup>	.85	*
CP	7.6	8.8	8.2	7.7	7.8	.98	ns
CF	26.8 <sup>a</sup>	24.5 <sup>ab</sup>	23.7 <sup>ab</sup>	23.1 <sup>b</sup>	22.3 <sup>b</sup>	1.8	*
EE	0.8 <sup>b</sup>	1.2 <sup>ab</sup>	1.6 <sup>a</sup>	1.7 <sup>a</sup>	1.8 <sup>a</sup>	0.42	*
NFE	52.9	53.3	53.9	54.0	53.9	1.3	ns
GE	3319	3230	3246	3259	3353	78	ns
DMD	37.2 <sup>c</sup>	40.2 <sup>b</sup>	40.7 <sup>b</sup>	41.3 <sup>ab</sup>	42.3 <sup>a</sup>	1.86	*
OMD	40.8 <sup>c</sup>	44.5 <sup>ab</sup>	43.9 <sup>b</sup>	45.5 <sup>ba</sup>	47.3 <sup>a</sup>	2.3	*

s.e = Standard error. Sol = significant observed level \* (P<0.05)

Table 2. Chemical composition, digestibility (g/100g DM) and GE(cal/g DM) of treatments at different times of post ensiling.

Item	Time periods (days)				s.e	sol
	20	40	60	80		
PH	8.13	8.02	8.07	8.16	0.08	ns
Ash	12.3	13.5	13.4	12.3	.74	ns
CP	5.5 <sup>b</sup>	8.7 <sup>a</sup>	8.9 <sup>a</sup>	9.0 <sup>a</sup>	.85	*
CF	27.6 <sup>a</sup>	24.9 <sup>b</sup>	23.8 <sup>bc</sup>	21.3 <sup>c</sup>	2.4	*
EE	1.2	1.4	1.5	1.6	0.4	ns
NFE	53.4	51.5	54.9	53.3	1.8	ns
GE	3297	3249	3267	3312	54	ns
DMD	35.4 <sup>b</sup>	41.1 <sup>a</sup>	42.7 <sup>a</sup>	41.5 <sup>a</sup>	1.4	*
OMD	38.0 <sup>b</sup>	44.1 <sup>a</sup>	45.4 <sup>a</sup>	45.0 <sup>a</sup>	1.8	*

s.e = Standard error. Sol = significant observed level \* (P<0.05)

**Conclusions** Based on the present study, whey could be used as a sole liquid or in combination with water for urea treatment of straw. The urea-whey treated straw should be ensiled for forty days to obtain higher digestibility. This may be a simple method for preservation and utilization of whey along with enrichment of straw in ruminant nutrition.

**Acknowledgments** This study was supported by the Animal Science Research Institute of I.R.Iran and Research Center for Natural Resources and Animal Production of Kerman Provinces, I.R.Iran.

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## Effect of fungal treatment on the chemical composition, *in vitro* digestibility and *in sacco* degradability of wheat straw

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**Introduction** Fungal treatment has been recently considered as a promising method for improving the nutritive value of straw (Zadrazil *et al.*, 1997). Several studies have been conducted to identify species of white-rot fungi for assessing their ability to improve the nutritive value of straw (Yamakamwa *et al.*, 1992). Since there are many species of fungi in nature, there is an interest in characterising of some species. The objectives of this experiment were to study the effect of five *Pleurotus* fungi on the chemical composition, *in vitro* digestibility and *in sacco* degradability of wheat straw and evaluate their effect in upgrading the nutritive value of lignocellulosic materials.

**Materials and methods** Wheat straw was chopped into 5-10 cm length and soaked in water for 24h, then it was pasteurised at 100°C for one hour. The straw was inoculated with spawns of five *Pleurotus* fungi (coded: P-21, P-30, P-41, P-60 and P-90) and packed in the plastic bags and incubated in a fermentation chamber at 25°C and 78 ± 5 relative humidity. After 21 days, the straws were removed from the fermentation chamber, air dried and used for chemical analysis, *in vitro* digestibility and *in sacco* degradability. The data obtained were analysed according to the complete randomised design model consisting of five treatments plus one control and four replicates.

**Results** Fungal treatment significantly ( $P < 0.05$ ) increased the CP content of the straw. More effect was observed from the P-41 and P-60 (3.5 and 3.4 g/100g respectively) compared to P-21, P-30 (3g/100g) and P-90 (2.7g/100g). All treatments significantly ( $P < 0.05$ ) reduced the NDF and ADF contents of the straw, however the ability of the fungi to degrade these components varied among the species. The ability of P-21 and P-90 were significantly ( $P < 0.05$ ) lower than the other species in decreasing the NDF and ADF contents. All treatments significantly ( $P < 0.05$ ) decreased the ADL content of the straw except for P-90. Fungal treatment also reduced the concentration of cellulose and hemicellulose ( $P < 0.05$ ) with different ability. All species of fungi incubated on wheat straw showed significantly ( $P < 0.05$ ) increased in the *in vitro* dry mater and organic mater digestibility. The degradability of DM and ADF at 48h incubation in the rumen, significantly ( $P < 0.05$ ) increased in fungal treated straw as compared to the control. Comparing the species of fungi, P-41 and P-51 caused the highest and the lowest degradability of ADF, respectively (Table 1). The degradability parameters including a (soluble fraction), b (not soluble but fermentable fraction) p (potential degradability) and c (constant rate) of all fungal treated samples were significantly ( $P < 0.05$ ) higher than the control. However, samples treated by P-41 and P-51 had the highest (20, 48.6, 43.9 and 0.044) and the lowest (16.3, 40.8, 37.2 and 0.037) degradability parameters respectively.

Table 1. Chemical composition, digestibility and degradability of untreated and fungal treated wheat straw<sup>#</sup>.

	Treatments					Control	s.e.
	2021	2030	2041	2050	2060		
OM	94.1	93.8	94.4	94.6	93.9	95.3	0.33
CP	2.8 <sup>b</sup>	2.9 <sup>b</sup>	3.2 <sup>ab</sup>	2.9 <sup>b</sup>	3.5 <sup>a</sup>	1.6 <sup>c</sup>	0.36
NDF	76.2 <sup>b</sup>	76.3 <sup>b</sup>	75.4 <sup>bc</sup>	75.4 <sup>bc</sup>	74.3 <sup>c</sup>	83.6 <sup>a</sup>	0.83
ADF	57.7 <sup>b</sup>	57.4 <sup>b</sup>	56.4 <sup>c</sup>	56.1 <sup>c</sup>	55.8 <sup>c</sup>	62.9 <sup>a</sup>	1.4
ADL	7.6 <sup>bc</sup>	7.1 <sup>d</sup>	7.3 <sup>cd</sup>	7.2 <sup>d</sup>	7.4 <sup>bcd</sup>	8.0 <sup>a</sup>	0.4
Cellulose	50.02 <sup>b</sup>	49.6 <sup>bc</sup>	49.1 <sup>bcd</sup>	48.8 <sup>cd</sup>	48.3 <sup>d</sup>	54.8 <sup>a</sup>	1.07
Hemicellulose	18.49 <sup>b</sup>	18.9 <sup>b</sup>	19 <sup>b</sup>	19.3 <sup>b</sup>	18.5 <sup>b</sup>	20.7 <sup>a</sup>	1.36
IVDMD	37.0 <sup>b</sup>	38.4 <sup>ab</sup>	40.3 <sup>a</sup>	31.6 <sup>bc</sup>	40.6 <sup>a</sup>	28.1 <sup>c</sup>	2.21
IVOMD	36.8 <sup>b</sup>	38.2 <sup>ab</sup>	40.2 <sup>a</sup>	32.0 <sup>bc</sup>	40.3 <sup>a</sup>	27.5 <sup>c</sup>	2.07
DM degradability	46 <sup>b</sup>	49 <sup>a</sup>	51 <sup>a</sup>	43 <sup>c</sup>	50 <sup>a</sup>	33 <sup>d</sup>	2.8
ADF degradability	34 <sup>c</sup>	41 <sup>b</sup>	46 <sup>a</sup>	30 <sup>c</sup>	42 <sup>b</sup>	22 <sup>d</sup>	1.3

<sup>#</sup> g/100g Dry mater basis. IVDMD = *in vitro* dry mater digestibility. IVOMD = *in vitro* organic mater digestibility. Means with the different superscripts within column are significantly ( $P < 0.05$ ) different.

**Conclusion** Fermentation of wheat straw by fungi could improve its nutritive value. The species P-30, P-41 and P-60 showed higher ability in improving the nutritive value of straw. The species P-41 seems to be more potent for upgrading of wheat straw.

**Acknowledgments** This work was supported by Animal Science Research Institute of I.R.Iran.

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# Structural characterization of low lignin (AC Assinobia) and high lignin (Normal) of oat hulls by Diffuse-Reflectance Fourier Transform Vibrational Infrared Spectroscopic analysis

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**Introduction** Oat hulls are a byproduct of the oat processing industry. Nutritionally, oat hulls are high in fiber, low in protein and are comparable to cereal straw as a feedstuff. As such, they are only suitable for ruminant feed. Due to the large supply, it is economically important to improve the nutritional qualities of this byproduct. Oat hulls contain hydroxycinnamic acids, which are covalently cross-linked to polysaccharides by ester bonds and to components of lignin mainly by ether bonds. These cross-links are a barrier to biodegradation and limit cell-wall degradability by rumen microorganisms. It is believed that these hydroxycinnamic acids are among the factors most inhibitory to the biodegradability of plant cell wall polysaccharides.

**Objective** The objectives of this study were to use a Diffuse-Reflectance Fourier Transform Infrared Spectroscopy to investigate structural characterization of low lignin (AC Assinobia) and high lignin (Normal) of oat hulls.

**Materials and Methods** Fourier transform vibrational infrared spectroscopy with diffuse-reflectance accessory, global source, MCT detector and KBr beam splitter were used for oat hulls structure analysis. The samples were placed in a micro-sampling cup and 256 scans were acquired at 4 cm<sup>-1</sup> resolution in the transmittance mode with subtraction of the KBr background. The spectra were then converted to the absorbance mode, baseline corrected and the region from 4000 to 500 cm<sup>-1</sup> displayed.

**Results and Discussion** Results show that the most bands of spectra of two oat hulls were qualitatively similar but probably quantitatively different. However, the aromatic ring region bands were significantly different. The bands between 3600 cm<sup>-1</sup> and 3000 cm<sup>-1</sup> for O-H stretch showed no visible difference in the bands between two hulls. The C-H stretching bands at 2920cm<sup>-1</sup> and 2855 cm<sup>-1</sup> were showed a similar pattern of fatty ester composition, representing the presence of lipid in both cell walls. The carbonyl stretch at 1730 cm<sup>-1</sup> was prevalent in the both hulls, but the absorbance was markedly higher in the commercial oat hulls. The aromatic ring band from 1660 to 1505 cm<sup>-1</sup> showed a obviously different pattern and ratio between the low lignin hull and the high lignin hulls, probably related to the different aromatic phenolic contents and protein. The commercial oat hulls showed a more prominent 1510 cm<sup>-1</sup> band, which has been identified in lignin, as due to ring stretching associated with aromatic C-O stretching. The bands from 1460 to 1020 cm<sup>-1</sup>, which are generally reflective of the carbohydrate content, gave similar absorbance in both hulls. In the region between 1000 and 800 cm<sup>-1</sup> a lower overall absorbance occurred in low lignin hulls. One sharp band at 898 cm<sup>-1</sup> occurred in the both hulls, but more prevalent in the commercial hulls. The 898 cm<sup>-1</sup> band probably arise from a ring stretch in cellulose. Results from this non-invasive vibrational infrared spectroscopic analysis have provided additional information to understand the functional group's structural barrier to biodegradation.

**Keyword:** FT-IR, Plant cell walls, Spectra, Functional group, Band, Biodegradation

## Study on chemical treatment of wheat straw with Norea alkali

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**Introduction** Feed shortage is the greatest obstacle to Iranian livestock industry. Historically, crop residues mainly cereal straw has been very important feed resources for ruminants during late summer and cold seasons in Iran. The digestibility and voluntary feed intake of these fibrous residues are quite low. The feeding value of fibrous co-products can be upgraded by chemical treatment (Galletti, 1991). Norea as a cheap locally produced alkali containing mainly lime and sulphur with higher solubility. Although the alkali is used for removing superfluous hair in human hygiene but it might also be effective for cell wall degradation in fibrous feeds. There was no any information on utilization of this alkali. Therefore, the reason for undertaking this experiment was to test this suggestion.

**Materials and methods** The effects of Norea treatment on nutritive value of wheat straw were studied in a completely randomised design with factorial arrangement of 2×2×8. The first main factor was the type of animal fitted with permanent fistula including three sheep and three goats. Water temperature of 20 and 60 °C was regarded as the second main factor. The last factor was Norea concentration in 8 levels of 0, 1.5, 3.0, 4.5, 6.0, 7.5, 9.0 and 10.5 g per 100 g wheat straw dry matter. The required Norea was dissolved in water, mixed and allowed to precipitate. The liquid phase was extracted and sprayed onto 3 kg well-collected straw samples and mixed by hands. The treated straws were stored for 48h in airtight condition under room temperature and then dried. Their chemical composition and rumen degradabilities were determined by *in sacco* method (1).

**Results** Cell wall (NDF) and ADF contents of treated straws decreased in accordance with increasing level of Norea. Organic matter content of wheat straw reduced following Norea application (about 2-3%) although, the proportion of lignin and hemicellulose remained unchanged. Rumen degradability of nutrients increased significantly ( $P<0.01$ ) under the effect of Norea treatment (Table 1). Similar results has been reported elsewhere following straw treatment by lime (Valizadeh 1994)

Higher water temperature led to higher dry matter and organic matter degradability in both animals. The rumen degradability of nutrients in goats was significantly ( $P<0.01$ ) higher than those in sheep.

**Table 1** Mean ( $\pm$ s.e) dry matter and organic matter degradability (%) of treated wheat straw with Norea\*

Type of animal	Level of Norea (%)	DM		OM	
		A	B	A	B
Sheep	0.0	43.7±0.6	44.1±0.5	45.1±0.7	45.3±0.7
	1.5	46.6±0.6	47.4±0.8	48.0±0.7	48.8±0.8
	3.0	48.1±0.8	49.3±0.6	49.3±1.0	50.1±0.7
	4.5	47.9±0.6	51.7±0.3	49.3±0.6	53.0±0.4
	6.0	49.3±0.8	52.4±0.9	50.6±0.6	52.9±0.8
	7.5	50.5±0.5	53.8±0.5	51.6±0.5	54.8±0.6
	9.0	52.4±0.4	54.6±0.5	53.1±0.6	55.5±0.5
	10.5	53.6±0.5	55.7±0.6	54.2±0.6	56.4±0.5
Goat	0.0	45.3±0.6	46.8±0.5	45.9±0.5	48.0±0.5
	1.5	47.5±0.7	48.2±0.5	48.4±0.7	48.8±0.4
	3.0	48.5±0.6	50.4±0.6	49.1±0.6	51.6±0.6
	4.5	49.3±0.5	52.3±0.5	49.9±0.6	52.9±0.3
	6.0	50.6±0.6	53.9±0.5	51.6±0.6	54.7±0.7
	7.5	51.6±0.9	55.7±0.6	52.5±1.2	56.7±0.4
	9.0	53.2±0.4	56.6±0.6	53.8±0.4	57.0±0.6
	10.5	54.7±0.7	56.9±0.7	55.0±0.7	56.8±0.6

\*A = Water temperature = 20 °C B = Water temperature = 60 °C DM = Dry matter OM = Organic matter

**Conclusions** It can be concluded that Norea is a stronger alkali than lime (Saadullah et al.1981). However, its safeness, effective concentration best method of practical and on farm application has to be studied in further researches. Economical consideration is an important issue related not only to the application of this alkali but, also to utilization of other chemicals in big scales and traditional animal farming in Iran.

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# Lipolysis and biohydrogenation of linoleic and linolenic acid *in vitro*: comparison of linseed products and grass

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**Introduction** With respect to human health, nutritional guidelines emphasise on increasing the ratio poly-unsaturated/saturated fatty acids (P/S) (> 0.7) and reducing the n-6/n-3 ratio (< 5). Beef is generally characterised by a low P/S ratio (0.1), while the n-6/n-3 ratio may vary between 2 and 10 depending on the feeding strategy. Hence, the provision of n-3 fatty acids by the diet is crucial for optimising the fatty acid composition of meat. However, PUFA are hydrolysed and subsequently hydrogenated in the rumen to more saturated fatty acids and intermediates that are absorbed from the intestinal tract and transported to the tissues. Lipolysis and hydrogenation may thus affect the meat fatty acid composition. For ruminants, important sources of linolenic acid (C18:3n-3) are linseed and fresh grass. Prior to use in animal nutrition, linseed needs physical treatment like extrusion or crushing. The aim of the present *in vitro* experiment was to study lipolysis and biohydrogenation of linoleic acid (C18:2n-6) and C18:3n-3 in fresh grass and extruded or crushed linseed products.

**Materials and methods** Three extruded (E1, E2, E3) and two crushed (C1, C2) linseed products (commercially available) and fresh grass were compared. The extent of lipolysis and biohydrogenation of C18:2n-6 and C18:3n-3 present in the triacylglycerol fraction of linseed and the polar lipid fraction of fresh grass was investigated by *in vitro* incubations. Each incubation contained 160 to 350 mg linseed (20 to 25 mg C18:3n-3) or 5 g fresh grass (12 to 13 mg C18:3n-3). Fresh grass was cut into pieces of 1 cm. Mixed rumen juice of three sheep, fed at maintenance on a hay/concentrate diet (65/35, wt/wt on DM basis), was taken before the morning feeding and filtered through a 1 mm sieve. *In vitro* incubations were carried out during 0, 2, 4 and 6h (and 24h for linseed), and fatty acids were extracted and analysed as described by Dohme *et al.* (2001). Lipolysis and biohydrogenation of C18:2n-6 and C18:3n-3 was calculated from their concentrations in the triacylglycerol fraction (TG) (or polar lipid fraction in case of fresh grass), and the free fatty acid fraction (FFA) before and after incubation according to the following formulas:

$$\text{Lipolysis} = (\text{TG}_0 - \text{TG}_t) / \text{TG}_0 \quad \text{Biohydrogenation} = 1 - (\text{FFA}_t / (\text{TG}_0 - \text{TG}_t + \text{FFA}_0))$$

with  $\text{TG}_0$ ,  $\text{TG}_t$ ,  $\text{FFA}_0$ ,  $\text{FFA}_t$  the concentration of linoleic or linolenic acid in the triacylglycerol or polar lipid fraction (TG), respectively the free fatty acid fraction (FFA) after 0, respectively t hours of incubation.

The extent of lipolysis and hydrogenation were analysed by ANOVA.

**Results** After 6h of incubation, lipolysis of both C18:2n-6 and C18:3n-3 was significantly higher for fresh grass than for linseed, and for extruded compared to crushed linseed (Table 1). After 24h of incubation, lipolysis of C18:3n-3 increased to more than 0.50 and 0.80 for crushed and extruded linseed respectively (data not shown). Independent of the substrate, the rate of hydrogenation of the free C18:2n-6 and C18:3n-3 is clearly higher than the rate of lipolysis, which confirms that lipolysis is the rate limiting process. Differences between substrates were also smaller for biohydrogenation than for lipolysis.

**Table 1** Mean values for the proportion of linoleic and linolenic acid lipolysed and biohydrogenated in two crushed (C1, C2) and three extruded (E1, E2, E3) linseed products and fresh grass after 6h of incubation (n=3)

		Lipolysis		Biohydrogenation	
		C18:2n-6	C18:3n-3	C18:2n-6	C18:3n-3
Crushed linseed	C1	0.028 <sup>a</sup>	0.041 <sup>a</sup>	0.375 <sup>a</sup>	0.625 <sup>a</sup>
	C2	0.104 <sup>a</sup>	0.074 <sup>a</sup>	0.606 <sup>a</sup>	0.720 <sup>ab</sup>
Extruded linseed	E1	0.286 <sup>b</sup>	0.296 <sup>b</sup>	0.803 <sup>b</sup>	0.885 <sup>c</sup>
	E2	0.334 <sup>b</sup>	0.349 <sup>b</sup>	0.839 <sup>b</sup>	0.924 <sup>c</sup>
	E3	0.373 <sup>b</sup>	0.377 <sup>b</sup>	0.790 <sup>b</sup>	0.865 <sup>bc</sup>
Fresh grass		0.580 <sup>c</sup>	0.740 <sup>c</sup>	0.524 <sup>ab</sup>	0.912 <sup>c</sup>
s.e.m.		0.041	0.042	0.097	0.049

<sup>a,b,c</sup> Means within columns with different superscript are significantly different (P<0.05)

**Conclusion** *In vitro*, linolenic acid is hydrolysed faster when present in fresh grass than in linseed, and faster in extruded than in crushed linseed. If this also occurs *in vivo*, rumen outflow of linolenic acid may be affected. This may in turn affect the availability of linolenic acid for tissue deposition.

**Acknowledgements** This research is supported by the EU-community (project QLRT-2000-31423 Healthy Beef). We are grateful to Andy Deprez for the technical assistance.

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## In vitro gas production, digestibility and estimated energy value of grass/fodder tree silages

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**Introduction** Use of grass/forage tree silages have prove to be a viable alternative for animal production in the tropics (Sol *et al.*, 2002a,b). It is also an adequate strategy to cope with seasonal fluctuation of biomass availability (both grass and forage tree). However, limited data is available on the nutritive value (e.g. digestibility, energy content) of this mixtures. Therefore, the objective of the present work was to evaluation the in vitro gas production, apparent digestibility and energy content of silages containing grass and forage trees.

**Materials and methods** Microsilages (1.8 L bottles) were prepared using *Pennisetum purpureum* grass alone (control) or mixed at 15, 30 and 45% inclusion of leaves and young branches (stem diameter < 5mm) of *Guazuma ulmifolia*, *Lysiloma latisiliquum*, *Piscidia piscipula*, *Albizia lebbbeck* and a mixture of the four previous species, resulting in 16 treatments. A 4% (of estimated DM) sugarcane molasses diluted 1:1 with water (w/v) was added to mixture. After 90 days silages were opened and samples taken and analyzed by the *in vitro* gas production technique (pressure transducer) with a N-rich media. The residues of fermentation were employed for digestibility estimates. The *in vitro* procedure was as follows: Rumen liquor from two cannulated cattle fed grass (*P. purpureum*) *ad lib.*, plus 3 kgDM concentrate (180 gCP/kgDM) was collected before morning feeding, 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<sub>2</sub>. Samples (0.5gDM) were incubated (4 replicates). Pressure and gas volume were measured at 3, 6, 9, 12, 15, 18, 21, 24, 30, 36, 48, 60, 72 and 96h. After 96h incubation, samples were filtered and dried (60 °C for 48h) and the residues employed to asses DMD by difference. The residues were then incinerated at 550 °C in order to calculate OMD, and the content of DOM in the DM (DOMDM). All digestibility estimations were corrected with the mean of four blanks. The ME content of samples was estimated as ME = %DOMDM \* 0.157.

Gas production profiles were fitted using a monophasic equation (Groot *et al.*, 1996).

**Table 1.** In vitro gas production profiles of grass/ forage tree mixtures

	Control	<i>L. latisiliquum</i>	<i>G. ulmifolia</i>	<i>P. piscipula</i>	<i>A. lebbbeck</i>	Mixture	0%	15%	30%	45%
TG	196 <sup>a</sup>	154 <sup>c</sup>	179 <sup>b</sup>	177 <sup>b</sup>	179 <sup>b</sup>	180 <sup>b</sup>	196 <sup>a</sup>	181 <sup>b</sup>	175 <sup>b</sup>	165 <sup>c</sup>
B	28.8 <sup>c</sup>	40.2 <sup>a</sup>	32.8 <sup>b</sup>	32.8 <sup>b</sup>	29.5 <sup>c</sup>	33.6 <sup>b</sup>	28.8 <sup>c</sup>	33.1 <sup>ab</sup>	34.7 <sup>a</sup>	33 <sup>b</sup>
C	1.92 <sup>a</sup>	1.57 <sup>c</sup>	1.59 <sup>bc</sup>	1.70 <sup>b</sup>	1.57 <sup>c</sup>	1.43 <sup>d</sup>	1.92 <sup>a</sup>	1.64 <sup>b</sup>	1.54 <sup>c</sup>	1.50 <sup>c</sup>
K	0.94	0.81	0.84	0.89	0.81	0.76	0.94	0.80	0.58	0.75
TG (ee)	5.25	5.589	4.483	4.933	3.609	4.189	5.25	3.84	2.88	3.7
B (ee)	1.12	2.25	1.31	1.39	0.97	1.33	1.12	1.09	0.92	1.2
C (ee)	0.091	0.062	0.054	0.067	0.046	0.041	0.091	0.05	0.03	0
R <sup>2</sup>	0.989	0.993	0.995	0.993	0.996	0.996	0.989	1	1	1
RSD	6.278	3.23	3.517	4.22	3.187	2.775	6.278	3.11	2	2.7

**Results** Gas production profiles are presented in Table 1. Inclusion of trees

TG: total gas (ml/gDM), B: time needed to produce half TG, c: dimensionless Factor to adjust sharpness of the curve, k: fermentation rate, all parameters estimated as Groot *et al.*, 1996; TG(ee), B(ee), C(ee); estándar error of equation parameters.

linearly reduced the amount of total gas as well a the rate of fermentation. The apparent *in vitro* digestibility (DMD, OMD) and Energy content of the silages was also reduced by the forage tree inclusion (Table 2). This reductions might be associated with the higher lignin content reported for this trees when compared with the grass (Lizarraga *et al.*, 2001).

**Table 2.** In vitro apparent digestibility (g/kg DM) and energy value (MJ/kg DM) of Grass/forage tree mixtures

	Control	<i>L. latisiliquum</i>	<i>G. ulmifolia</i>	<i>P. piscipula</i>	<i>A. lebbbeck</i>	Mixture	sem	Control	15%	30%	45%	sem
DMD	639±16a	459a	509b	538c	580d	534bc	9	639±23c	544b	530b	498a	10
OMD	649±16a	463 <sup>a</sup>	522b	549c	586d	538bc	9	418±23a	494b	560b	496b	10
ME	9.4±0.5c	6.8 <sup>a</sup>	7.6ab	7.9bc	8.5cd	7.9bc	0.3	9.4±0.7	8.1	7.8	7.3	0.3

**Conclusion** Inclusion of tree in tropical grass silages reduced apparent *in vitro* DMD, OMD and estimated ME value. However, intake and animal performance must be taken in account for a better assessment of their nutritional value.

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# Estimation of the nutritive value of silage from grass (*Pennisetum purpureum*) and forage tree mixtures

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**Introduction** Silage is recognized as a viable option to cope with seasonal fluctuations in forage availability. Due to the reduced nutrient contents (N and soluble CHO's) of tropical grasses, the resulting silage is usually of low quality. This factor plays a major role in the adoption of this technology by farmers in developing countries. The inclusion of forage trees to improve the nutritive value of silage is seen as a viable alternative, as forage trees are locally available and farmers are increasingly implementing its use as supplement. Silage resulting from grass/tree mixtures might allow reduced use of conventional grain-based supplement without jeopardizing animal performance (Sol *et al.*, 2002a,b). However, few data is available about adequate inclusion levels of forage tree and grass for silage making. Thus, the objective of the present work was to assess changes in silage quality associated with the inclusion level of forage tree.

**Material and methods** Microsilages (1.8 L bottles) were prepared using *Pennisetum purpureum* grass alone (control) or mixed at 15, 30 and 45% inclusion level of *Guazuma ulmifolia* (Pixoy), *Lysiloma latisiliquum* (Tzalam), *Piscidia piscipula* (Jabín), *Albizia lebbek* (algarrobo) and a mixture of the four previous species, resulting in 16 treatments. Each treatment was replicated five times. A 4% (of estimated DM) sugar cane molasses was added to mixture, molasses was diluted 1:1 with water (w/v) to facilitate mixing. After 90 days silages were opened and samples taken to evaluate chemical composition (DM, CP, NDF, ADF), condensed tannins (CT) (Price *et al.*, 1978), pH and fermentation products (lactic, acetic, and butyric acids). *In vitro* DM apparent digestibility (IVDMD) was estimated by difference between the residue and incubated sample (96h) from an *in vitro* gas production test (Theodorou *et al.*, 1994) which was run simultaneously. Specie, inclusion and their interaction were included in the statistical analysis, together with a surface response analysis. However, only main effects are presented due to space constraints.

**Results** The chemical composition, pH and IVDMD at silage opening (90 days) averaged by type of forage tree and inclusion level are presented in Table 1. Inclusion of forage tree into the silage increased its CP content with relatively small changes in fermentation characteristics (Lactate, Acetate, pH). Inclusion of forage trees caused a reduction on IVDMD probably due to their high lignin content as reported by Lizarraga *et al.* (2001). *L. latisiliquum* caused the greatest reduction ( $P<0.05$ ) on IVDMD. A silage containing all four tree species was of similar quality to those elaborated with a single tree specie.

**Conclusion** Inclusion of forage trees into grass silage allows the farmer to obtain a product of improved nutritive value (CP). There is no difference in the silage obtained when using single tree specie or a mixture of forage trees. Therefore, farmers do not need to select a specific tree to incorporate into the silage, as a mixture of trees can be used, thus, simplifying the process of silage making.

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**Table 1.** Chemical composition (g/kgDM) of *Pennisetum purpureum* and forage tree silage

	DM	CP	NDF	ADF	CT
Control	278	72	652	421	9
	±13 <sup>ab</sup>	±15	±34	±19	±9 <sup>ab</sup>
Tzalam	309 <sup>b</sup>	77	648	399	25 <sup>ab</sup>
Pixoy	249 <sup>a</sup>	85	614	399	12 <sup>ab</sup>
Jabín	263 <sup>a</sup>	90	616	392	08 <sup>a</sup>
Algarrobo	271 <sup>a</sup>	95	597	388	19 <sup>ab</sup>
Mixture	305 <sup>b</sup>	84	598	389	14 <sup>ab</sup>
s.e.m.	8	8	19	11	5
Inclusion level					
Control	278	72	652	421	9
	±28	±8 <sup>ab</sup>	±25 <sup>b</sup>	±14 <sup>b</sup>	±9
15%	270	72 <sup>a</sup>	645 <sup>b</sup>	409 <sup>b</sup>	12
30%	281	86 <sup>b</sup>	615 <sup>ab</sup>	390 <sup>ab</sup>	13
45%	287	99 <sup>c</sup>	584 <sup>a</sup>	382 <sup>a</sup>	22
s.e.m.	12	4	11	6	4

CT: condensed tannins, S.e.m.: for control and treatments are provided separately.

**Table 2.** Fermentation characteristics (g/kgDM) of *Pennisetum purpureum* and forage tree silage

	pH	LA	AA	BA	IVDMD
Control	3.9	45	33	10	639
	±0.07 <sup>b</sup>	±12 <sup>ab</sup>	±8 <sup>ab</sup>	±6	±16e
Tzalam	3.6 <sup>a</sup>	25 <sup>a</sup>	21 <sup>a</sup>	3	459 <sup>a</sup>
Pixoy	4.0 <sup>b</sup>	52 <sup>b</sup>	34 <sup>ab</sup>	9	509 <sup>b</sup>
Jabín	4.1 <sup>c</sup>	63 <sup>b</sup>	29 <sup>ab</sup>	5	538 <sup>c</sup>
Algarrobo	4.0 <sup>b</sup>	58 <sup>b</sup>	39 <sup>b</sup>	3	580 <sup>d</sup>
Mixture	3.6 <sup>a</sup>	49 <sup>b</sup>	22 <sup>a</sup>	0	534 <sup>bc</sup>
s.e.m.	0.04	7	5	3	9
Inclusion level					
control	3.9	45	33	10	639
	±0.1 <sup>b</sup>	±17	±11	±6	±23 <sup>c</sup>
15%	3.9 <sup>a</sup>	59	29	6	544 <sup>b</sup>
30%	3.8 <sup>a</sup>	44	27	3	530 <sup>b</sup>
45%	3.9 <sup>a</sup>	45	31	4	498 <sup>a</sup>
s.e.m.	0.1	7	5	3	10

NH<sub>3</sub>-N as % of total N, pH: log units, LA: lactic acid, AA: acetic acid, BA: Butyric acid, IVDMD : *in vitro* dry matter apparent digestibility, S.e.m.: for control and treatments are provided separately.

## Ruminal N degradability of fresh, wilted and ensiled temperate forages

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**Introduction** Spring excess of temperate pastures are usually ensiled by Uruguayan farmers. The purpose of this work was to study changes in the ruminal N degradability during wilting and silage fermentation of farm forages in Uruguay.

**Material and Methods** Seven cultivated pastures, mixtures of grasses (*Festuca arundinacea*, *Lolium multiflorum*) and legumes (*Trifolium repens*, *Trifolium pratense*, *Medicago sativa*) were selected from dairy Uruguayan farms (34° latitude S, 55° longitude W). Each pasture was sampled during the following steps of the silage making process: fresh forage (F), wilted forage (W) (7.8±1.2 h) and silage (S) (round bales of 700 kg). Mean composition is given in table 1. Samples (n=21) were lyophilised and analysed for crude protein (CP), soluble protein in buffer phosphate (SP), neutral and acid detergent insoluble nitrogen (NDIN and ADIN) and incubated *in situ* for 0, 2, 4, 8, 12, 24, 48 and 72 h in nylon bags (ANKOM Tech. Corp.), in three cows. Two series of incubations were conducted for each sample. Incubated bags were washed, dried (80°C, 48 h), analysed for N. Disappearance was fitted by non-linear regression for each sample and cow to the model proposed by Ørskov and McDonald (1979). Effective degradability was estimated using particulate outflow rates ( $kp$ ) of 0.03/h (ED03) and 0.06/h (ED06). Effect of treatment (F, W and S) on chemical N fractions and degradation characteristics was analysed by ANOVA considering animal, pasture and treatment effects. Means were compared by orthogonal contrasts.

**Table 1** Chemical composition of fresh forage (F), wilted (W) and silage (S) (g/kg DM)

	F	W	S
DM (g/kg)	195.8	372.4	380.6
OM	919.7	918.3	910.4
NDF	495.5	515.5	555.3
ADF	371.6	387.7	434.5

DM: dry matter; OM: organic matter; NDF: neutral detergent insoluble nitrogen; ADF: acid detergent insoluble nitrogen

**Results** Table 2 shows N fractions and degradation characteristics in each step. Wilting lead to a reduction in the ED06, as a result of an increase in the non-soluble degradable fraction ( $b$ ) with a decrease in fractional degradation rate ( $k_d$ ), but chemical N fractions were not affected. Fermentation and storage transformed part of the nitrogenous components from the  $b$  fraction to the soluble ( $a$ ) and undegradable ( $u$ ) fractions. This generated an increment in ED03 and ED06 (although only ED06 was significantly affected). These changes were accompanied chemically by an increase in SP and ADIN. The increase in  $a$  and SP fractions may be evidence of protein degradation and deamination in the silos. The increment in the  $u$  fraction was accompanied by the increment in ADIN and may indicate an increase of protein linked to cell walls due to Maillard reactions.

**Table 2** Effects of wilting and ensiling on chemical nitrogen fractions and rumen N degradation characteristics of temperate grass-legume forages.

Item	F	W	S	SEM	F vs W <sup>1</sup>	(F+W) vs S <sup>1</sup>
CP (g/kg DM)	117.4	115.2	115.1	3.620	0.678	0.797
SP <sup>2</sup>	0.273	0.274	0.443	0.029	0.987	<0.001
NDIN <sup>2</sup>	0.171	0.204	0.206	0.018	0.232	0.423
ADIN <sup>2</sup>	0.095	0.103	0.136	0.010	0.586	0.014
N degradation characteristics						
$a$	0.454	0.409	0.597	0.017	0.075	<0.001
$b$	0.383	0.424	0.219	0.014	0.038	<0.001
$u$	0.164	0.167	0.185	0.006	0.666	0.007
$kd$ (h <sup>-1</sup> )	0.133	0.111	0.110	0.006	0.020	0.155
ED03	0.765	0.739	0.765	0.006	0.002	0.064
ED06	0.717	0.680	0.735	0.007	<0.001	<0.001

F: fresh forage; W: wilted forage; S: silage;  $a$ : soluble fraction;  $b$ : non-soluble degradable fraction;  $u$ : undegradable fraction;  $k_d$ : fractional degradation rate of fraction  $b$ . SEM: standard error of the mean. <sup>1</sup>: Probability of the orthogonal contrast. <sup>2</sup>: expressed as decimal proportion of CP.

**Conclusions** The largest changes during ensiling were the increase in  $a$  fraction and decrease in the  $b$  fraction for N, produced during the fermentation and storage. These changes were accompanied by an increase in SP and ADIN.

**Acknowledgments** This work was supported by CSIC (UdelaR). A. Curbelo and C. Soto developed their work as CIDEDEC scholarship students (Facultad de Veterinaria, UdelaR)

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# Chemical composition and *in vitro* dry matter digestibility of parasitic plants reflect that of indigenous browse trees

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**Introduction.** Mistletoes are common parasitic plants that attach on branches of *Acacia* species, *Boscia albitrunca*, *Ziziphus mucronata* and other trees of semi-arid Botswana. These plants form an interesting alternative and additional feed resource, which could increase both mineral and protein intake of ruminants. Previous studies (Madibela *et al.*, 2000, 2002) have shown that these parasitic plants have high crude protein and mineral levels than what is expected of natural grasses. The hypothesis is that the higher the nutritive value of host browse trees the higher it is in the parasitic plants.

**Materials and methods.** Samples (the first 15 to 20 cm of leaves and stem) of *Tapinanthus lugardii*, *Erianthenum ngamicum*, *Viscum rotundifolium* and *V. Verrucosum* were collected from January to June in 1999 from Sebele Station. Leaves and terminal shoots of host browse trees were also sampled. Chemical analysis was made for ADF, ADL, NDF, NDF-n, minerals and nitrogen. IVDMD was also determined. General linear models (GLM) procedure was used to test the effects of host browse plant on chemical composition and IVDMD.

**Results and discussion.** Browse species did not have any effect ( $P>0.05$ ) on chemical composition and IVDMD of *T. lugardii*. ADF in *E. ngamicum* was highest ( $P<0.05$ ;  $34.02\pm 1.31\%$  DM) in leaves and stems harvested from *Acacia fleckii* and the least ( $26.64\pm 3.21\%$  DM) from those harvested from *A. tortilis*. Samples from *V. verrucosum* parasiting *A. tortilis* and *Dichrostacyc cinerea* had high ( $P<0.05$ ) levels of CP ( $17.79\pm 0.94$  and  $17.27\pm 0.94\%$  DM respectively) than those parasiting *A. robusta* ( $14.06\pm 0.94\%$  DM). Ca level of samples of *V. verrucosum* harvested from *A. robusta* were however higher ( $P<0.01$ ;  $2.06\pm 0.14\%$  DM) than those harvested from either *A. tortilis* ( $1.42\pm 0.14\%$  DM) or *D. cinerea* ( $1.40\pm 0.14\%$  DM). P level of *V. verrucosum* was high ( $P<0.01$ ) in samples from *A. tortilis* ( $0.21\pm 0.02\%$  DM) and low ( $0.13\pm 0.02\%$  DM) from *A. robusta*, and *D. cinerea*. Levels of CP of *V. rotundifolium* were higher ( $P<0.001$ ;  $19.58\pm 1.05\%$  DM) from samples harvested from *Boscia albitrunca* and lowest ( $11.44\pm 1.05\%$  DM) from those harvested from *Maytenus senegalesis*. Phosphorous in leaves and stem of *V. rotundifolium* harvested from *Ziziphus mucronata* was highest ( $P<0.05$   $0.20\pm 0.01\%$  DM) and those from *B. albitrunca* and *M. senegalesis* the least ( $0.16\pm 0.01$  and  $0.15\pm 0.01\%$  DM, respectively). Plotting CP, Ca, P and IVDMD over a six months period showed that nutrients contents of parasitic plants follow closely those of their hosts except for Ca (Figure 1). Different host browse plants confer variable chemical composition levels to similar individuals parasitic plants parasitizing different hosts, partly due to variation in the supply of nutrients from the host sap. In particular the level of nutrients from *Viscum* species reflects those of the browse probably indicating a unique relationship between these plants and their hosts.

■ CPbr    × IVDMDbr    ■ CPp    × IVDMDp  
◆ Cabr    ▲ Pbr    ◆ Cap    ▲ Pp

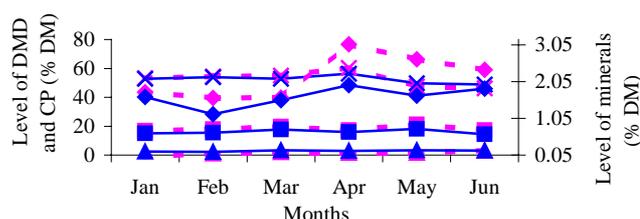


Figure 1. Nutritional attributes of browse trees and parasitic plants over a period of six months

**Conclusions** CP, Ca, P content in *V. verrucosum* and *V. rotundifolium* reflects those of different host plants. This may be due to differences in nutrient supply by the host browse plants. The higher the nutritive value in the browse the higher it is in the parasitic plants. The levels of CP, P and IVDMD of parasitic plants followed the same pattern as that of browse over the six-months sampling period. Parasitic plants can be harvested for animal feeding if the host browse of high nutritive value has physical impediments obstructing grazing by animals.

**Acknowledgements** This work was funded by Botswana's Ministry of Agriculture.

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## Chemical composition of some feedstuffs in East Azerbaijan

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**Introduction** In Iran, East Azerbaijan province is the most important farm animal production area and alfalfa hay, barley grain, wheat straw are the most common ingredients of animal rations. Organic matter and minerals content of forages and grains can be influenced markedly by climate, soil and fertilizer treatment, growth stage and agronomic factors in a given area. Therefore, it is necessary that the chemical composition of forages and grains, which are produced under climate and agronomic condition of Azerbaijan, for using in formulating balance rations, were determined. The objectives of present study were to determine chemical composition and some macro elements of alfalfa hay, barley grain and wheat straw in Azerbaijan.

**Materials and methods** Using Probability Stratified Random Sampling, East Azarbyjan province was classified into 30 agricultural zones. In each zone, several villages were selected randomly. Alfalfa hay (AH), wheat straw (WS) and barley grain (BG) was subjected to sampling according to scientific methods in 124 villages. Crop products of 3-to5 farmers were subjected to sampling in each village. The samples of AH (second cut), BG and WS was taken from stac or depot. All samples were subjected to preliminary drying and grounding. Equal amounts of samples belonging to villages of a given zone, were mixed and then a new composite sample resulted for a given zone. In this way, 30, 26 and 26 samples was resulted for AH, WS and BG respectively. All samples were then grounded through a 1 mm screen prior to chemical analysis. Dry matter (DM), crude protein (CP), ether extract (EE) and Ash contents of feeds according to AOAC (1990) and acid detergent fibre (ADF) and neutral detergent fibre (NDF) according to Goerring and Van Soest (1970) procedure were measured. The GLM procedure of SAS was used for statistical analysis of data.

**Results** In most cases, the mean values for chemical composition in AH, WS and BG of Azerbaijan were significantly ( $P<0.01$ ) different from those reported by NRC (2001). Means DM of AH, WS and BG were significantly higher than that in NRC. Mean CP content of AH and WS was as much as 17.5 and 14.6 % less than that in NRC. The CP content of BG was as much as 9.5 % higher than that in NRC. In case of EE, significant differences was only found between BG of Azerbaijan and NRC. NDF content of AH and BG was as much as 12.5 and 24% more than that reported by NRC, respectively. Mean ADF content of WS and BG was less and more than that reported by NRC, respectively. The highest and lowest NFC values were found in BG and WS, 140 and 568 g/kg DM respectively. Mean ash values of AH and WS in comparisons to the reported ash content for same feeds by NRC, showed significant differences ( $P<0.01$ ). The CV of mineral contents, especially Na, was relatively high in among samples of a given feed (11 to 115 %). Ca content in WS and BG was as much as 53.8 and 42.5 % more than that in NRC. P content in AH and BG in comparison to the reported value for same feeds by NRC, was lower and higher respectively. Mg content in all three studied feeds was significantly higher than that reported for same feeds in NRC. K content of the studied feeds, except, in comparisons to the reported values in NRC, didn't show significant difference. Finally, Na content in all three studied feeds was not significantly different from Na of the same feeds in NRC.

**Table1** Mean and standard deviation (in parentheses) of chemical composition and some macro elements of alfalfa hay, wheat straw and barley grain in Azerbaijan (g/kg DM)

Item	AH (n=30)	NRC <sup>1</sup>	Sign	WS (n=26)	NRC	Sign	BG (n=26)	NRC	Sign
DM	924(7)	903	**	948(7)	927	**	925(3)	910	**
CP	159(11)	192	**	41(5)	48	**	137(15)	124	**
EE	24(2)	25	NS	15(0.8)	16	ns	20(1)	22	**
NDF	477(39)	416	**	703(55)	730	ns	279(53)	208	**
ADF	347(51)	328	ns	455(31)	494	**	81(12)	72	**
NFC <sup>2</sup>	242(32)	-	-	140(52)	-	-	534(53)	-	-
Ash	98(10)	110	**	101(15)	76	**	30(5)	29	ns
Ca	13.1(3.3)	14.7	ns	5.4(1.3)	3.1	**	1.3(0.3)	0.6	**
P	0.72 (0.15)	2.8	**	1.23(8)	1	ns	5(1.2)	3.9	**
K	23.8(5)	23.7	ns	14(4.1)	15.5	ns	7(0.8)	5.6	**
Mg	8.4(3.7)	2.9	**	4.1(1.1)	1.4	**	3.8(1.1)	1.4	**
Na	0.73(55)	1.0	ns	0.89(1)	1.2	ns	0.27(0.18)	0.2	ns

1-The reported data for the same feeds in NRC. 2-Non-fibre carbohydrate, calculated by  $100 - (NDF\% + CP\% + EE\% + Ash\%)$  formula (NRC 2001)

**Conclusion** The overall result showed that the studied chemical composition and mineral content in three feeds of azerbaijan had considerable differences with data reported for these feed by NRC. Therefore, using NRC data in formulating rations which is more common in animal production system in Azerbaijan, is inaccurate and the producers must be consider the finding of present study and others in this respect.

**Acknowledgments** This research project has been supported by Grant No.NRCI 3953 of National Research Projects and with the support of National Research Council of Islamic Republic of Iran.

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## Nutritive evaluation of heat-treated cottonseed with *in vitro* gas production

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**Introduction** Biological methods are more meaningful since microorganisms and enzymes are more sensitive to factors influencing the rate and extent of digestion than are chemical methods (Getachew and et al,1997). Gas measurements provides a useful data on digestion kinetics of both soluble and insoluble fractions of feedstuffs. The objective of this experiment was to determine nutritive value evaluation of heat-treated cottonseed with *in vitro* gas production.

**Materials and methods** Whole cottonseed were obtained within the Iran and delinted (DCS) was heated in a cooker of commercial oil mill, and the following treatments were prepared: 130°C and 0 minute steep (1300), 130°C and 10 min steep (1310), 130°C and 20 min steep (1320), 140°C and 0 min steep (1400), 140°C and 10 min steep (1410), 140°C and 20min steep (1420), 150°C and 0 min steep (1500), 150°C and 10 min steep (1510), 150°C and 20 min (1520). The *in vitro* gas production technique (Theodorou and et al,1994) was used to determine the gas production. Gas production after 48 h incubation (GAS48) and at the end of 24 h incubation gas production (GAS24), ammonia-N and *in vitro* true digestibility (IVTD) were determined. The IVTD was calculated as the weight of substrate incubated minus the weight of residue after neutral detergent treatment. Data were analyzed using GLM procedure of SAS.

**Results** Mean gas produced at 48 h, 24 h, N-NH<sub>3</sub> and IVTD of cottonseed and heat-treated cottonseeds are given in table 1. There is significant differences between GAS48, GAS24, N-NH<sub>3</sub> and IVTD (p<0.05). Heating caused a decrease of GAS24 as heating temperature increase from 130°C to 140°C and N-NH<sub>3</sub> concentration increase in 140°C and reduce in 150°C (p<0.05). GAS24 and IVTD increase as the amount of heat exposure increased from 0 to 20 min (p<0.05). There isn't significant differences between GAS48 as temperature and heat exposure increase. There is negative relation between GAS24 and N-NH<sub>3</sub> concentration.

**Table 1** Mean gas produced at 48 h, 24 h, N-NH<sub>3</sub> and IVTD of cottonseed and heat-treated cottonseeds

	UCS	1300	1310	1320	1400	1410	1420	1500	1510	1520	SEM
Gas produced (ml/48h/gDM)	119.9 <sup>b</sup>	161.4 <sup>a</sup>	133.4 <sup>ab</sup>	151.1 <sup>ab</sup>	151.1 <sup>ab</sup>	149.6 <sup>ab</sup>	146.3 <sup>ab</sup>	155.5 <sup>a</sup>	148.2 <sup>ab</sup>	151.4 <sup>a</sup>	13.5
Gas produced (ml/24h/gDM)	83.0 <sup>e</sup>	110.0 <sup>bc</sup>	103.4 <sup>dc</sup>	122.3 <sup>a</sup>	100.72 <sup>d</sup>	98.6 <sup>d</sup>	125.0 <sup>a</sup>	85.0 <sup>e</sup>	98.1 <sup>d</sup>	112.8 <sup>b</sup>	3.9
N-NH <sub>3</sub> (mg/l)	330.4 <sup>a</sup>	285.6 <sup>b</sup>	298.7 <sup>b</sup>	246.4 <sup>f</sup>	255.3 <sup>ef</sup>	276.7 <sup>dc</sup>	268.3 <sup>de</sup>	275.3 <sup>dc</sup>	280 <sup>dc</sup>	259 <sup>ef</sup>	6.3
IVTD(%)	76.1 <sup>a</sup>	40.8 <sup>e</sup>	46.7 <sup>bc</sup>	47.0 <sup>bc</sup>	46.5 <sup>bc</sup>	47.5 <sup>bc</sup>	50.1 <sup>b</sup>	45.3 <sup>cd</sup>	45.1 <sup>cd</sup>	42.8 <sup>d</sup>	2.0

Means in the same row with unlike supercripts differ (p<0.05)

**Conclusions** The high rumen degradability of cottonseed protein caused in increased rumen ammonia *in vitro*. Heat temperature and duration can reduce rumen protein degradability in cottonseed. Heat processing of cottonseed alters gas production parameters *in vitro*.

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## Changes in water soluble carbohydrate content during the day in Lucerne and Fescue cut in autumn

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**Introduction** The content of water-soluble carbohydrates (WSC) in plants is variable, and depends on plant species and environment conditions. This content may be the limitation for the fermentation during silage making and also for microbial synthesis in rumen. The objective of this study was to measure the variations of the WSC in temperate forages, during autumn, in different moments of the day and in different parts of the plant.

**Material and Methods** The study was developed in Uruguay (34° latitude S, 55° longitude W) during autumn 2002. Two different cultivated pastures: Fescue (*Festuca arundinacea*) and Lucerne (*Medicago sativa*), were sampled at April 3 (3/4), May 8 (8/5) and May 30 (30/5). Chemical composition of the pastures is presented in Table 1.

**Table 1** Chemical composition of the forages in each day (g/kg DM)

	Lucerne			Fescue		
	3/4	8/5	30/5	3/4	8/5	30/5
kg DM/ha	2968	1465	2083	2623	1870	3356
DM (g/kg)	219.2	149.9	196.5	154.3	157.3	174.8
OM	925.0	906.7	911.7	878.2	875.5	874.5
CP	163.6	242.2	219.5	192.8	197.9	214.0
ADF	362.3	252.1	264.0	334.7	293.8	305.7

DM: dry matter; OM: organic matter; CP: crude protein; ADF: acid detergent fibre

In each day, pastures were sampled in 3 different moments: at 9 h, 13 h and 17 h. Samples were dried at 60°C. A part was separated in leaves and stems and the rest was analysed as whole plant. Whole plant, leaves and stems were analysed for WSC (Yemm and Willis, 1954). Results of WSC were analysed for Lucerne and Fescue by ANOVA. Means between days, hours and part of the plant for each specie were compared by LSD test.

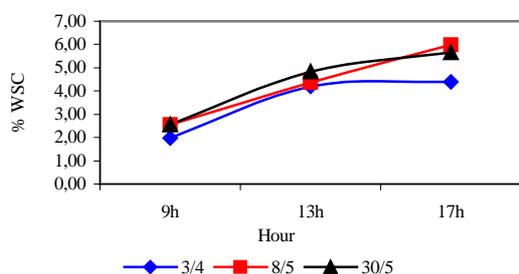
**Results** Table 2 shows WSC for Lucerne and Fescue. These contents were low particularly for fescue in the morning, but they increased during the day (Figures 1 and 2). They were higher at midday and afternoon because of plant photosynthesis. Stems had higher contents than leaves in Lucerne, but in fescue there were not significant differences between parts of the plant.

**Table 2** WSC (g/kg DM) in different days, hours and of different parts of Lucerne and Fescue.

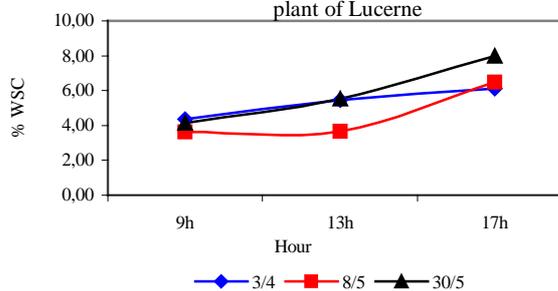
	day				hour				part				sem
	3/4	8/5	30/5	P	9	13	17	P	leaves	stems	whole plant	P	
lucerne	53.40 <sub>b</sub>	44.55 <sub>c</sub>	63.79 <sub>a</sub>	<0.001	40.59 <sub>c</sub>	52.49 <sub>b</sub>	70.99 <sub>a</sub>	<0.001	52.47 <sub>b</sub>	57.10 <sub>a</sub>	52.52 <sub>b</sub>	0.049	1.41
fescue	34.51 <sub>b</sub>	42.30 <sub>a</sub>	41.87 <sub>a</sub>	<0.001	24.48 <sub>c</sub>	40.61 <sub>b</sub>	51.13 <sub>a</sub>	<0.001	38.72	39.28	39.03	0.372	1.07

Different letters in a same row indicate a significant difference (P<0.05)

**Figure 1** Variation during the day in WSC in whole plant of Fescue



**Figure 2** Variation during the day in WSC in whole plant of Lucerne



**Conclusions** WSC increased during the day for both species, they were significantly higher in the afternoon. Contents were low for fescue, particularly in the morning.

**Acknowledgments** This work was supported by International Foundation for Sciences. N.Errandonea developed his work as CIDEDEC scholarship student (Facultad de Veterinaria, UdelaR)

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## Pinpointing the lowest protein diet for young male broilers

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**Introduction:** Protein is one of the most expensive portion of a broiler chicken diet. Overfeeding of protein may reduce broiler production profit as well as polluting soil through extra nitrogen excretion. Many attempts have been made to reduce dietary crude protein (CP) level with no adverse effect on broiler performance, as a result protein per se is no longer a requirement for growing chicken. Controversial results have been published with regard to lowering dietary CP level. The purpose of these studies was to pinpoint out the lowest possible dietary protein level when supplemental indispensable amino acids are maintained in a practical corn-soy diet.

**Materials and methods:** A duplicate of corn and soybean meal (as sole sources of intact protein) were analyzed for AA content by ion exchange chromatography (LKB, Biochrom, Cambridge Science Park, UK). One hundred day old Ross broiler chicks raised on a 4 m<sup>2</sup> floor pen and fed a pretest corn-soy diet (3200Kcal MEn/Kg and 23% CP). All commercial management practices were maintained. At the start of each experiment following 4-h deprivation of feed, the chicks weighed singly, placed in 3 weight categories, wing banded and every 3 chicks were randomly assigned to each pen (m<sup>2</sup>) in order to have similar pen weight. In experiment 1, four dietary treatments contained 17, 19, 21 and 23% CP were randomly assigned to pens in order to have 5 replicates per treatment and fed to chicks from 9 to 16 days of age. All diets in both experiments were isocaloric (3200 Kcal MEn/Kg) and supplemented with appropriate levels of synthetic amino acids to match NRC (1994) recommendation. Based on the results obtained from the first EXP, EXP 2 was designed similar to EXP 1 but the protein levels changed to 18, 19, 20 and 23% and were fed to chicks from 6 to 16 days of age. Data from both experiments were separately analyzed by one – way ANOVA for completely randomized design using General Linear Models procedure of SAS software (SAS Institute, 1998). The treatment means differences were evaluated using Duncan's multiple range test.

**Results:** Results of both experiments are shown in Table 1. In the first experiment, reducing dietary CP up to 19% did not have a significant effect on broiler performance as compared to birds fed control diet, but lowering the dietary CP to 17% had significantly (P<0.05) reduced weight gain (by 5.91 g/b/d), feed intake (by 3.72 g/b/d) and increased feed conversion ratio (0.24 unit). In EXP 2, chicks fed 18% CP diet as compared to 23% had a significantly lower weight gain (by 4.62g/b/d) and higher feed conversion (by 0.34 unit). The performance of broiler chicks fed other diets was not different from those fed a control diet. Garcia et al (2000) reported lowering dietary CP content from 24 to 17% with no addition of appropriate amino acid levels resulted in a decrease broiler chicks weight gain by 20% and increase in feed conversion ratio by 13%. Our results revealed that 19% CP is the lowest possible CP level in a practical broiler starter diet with no adverse effect on chick performance when all indispensable amino acids are maintained at the NRC-1994 recommendation level.

**Table 1** Effect of dietary protein concentration on broiler chicks performance\*

Protein (%)		Weight gain	Feed intake	Feed conversion
Total	Intact	g/bird/day	g/bird/day	g:g
Experiment 1: 9 to 16 days of age				
23	22.7	25.77 <sup>a</sup>	37.94 <sup>ab</sup>	1.481 <sup>a</sup>
21	19.9	23.40 <sup>a</sup>	39.80 <sup>ab</sup>	1.723 <sup>a</sup>
19	16.7	26.51 <sup>a</sup>	40.71 <sup>a</sup>	1.538 <sup>a</sup>
17	13.3	19.86 <sup>b</sup>	34.22 <sup>b</sup>	1.719 <sup>a</sup>
Pooled SEM		1.11	1.80	0.092
Experiment 2: 6 to 16 days of age				
23	22.7	23.18 <sup>a</sup>	30.76 <sup>b</sup>	1.344 <sup>c</sup>
20	18.3	23.20 <sup>a</sup>	36.16 <sup>a</sup>	1.559 <sup>ab</sup>
19	16.7	21.11 <sup>a</sup>	30.68 <sup>b</sup>	1.458 <sup>bc</sup>
18	15.1	18.56 <sup>b</sup>	31.18 <sup>b</sup>	1.681 <sup>a</sup>
Pooled SEM		0.886	1.363	0.068

\* In each experiment means within a column with different superscript differ significantly (P<0.05)

**Conclusion:** The results of these experiments indicating that the performance of broilers fed a practical corn – soy diet containing 19% CP or higher and supplemented with appropriate amounts of indispensable amino acids is similar to those fed a diet with 23% CP.

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# The effect of natural zeolite and bakery waste on performance and serum parameters of broiler chickens

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**Introduction** Use of bakery waste in animal nutrition is a way to reduce feed cost and environmental pollution. Large quantities of bakery waste are produced in Iran. It is possible to replace wheat with bakery waste in poultry rations. Bakery waste has a considerable moisture and carbohydrate and might be polluted with mold and mycotoxins. The presence of mycotoxins in ration may lead to increase mortality and decrease performance of broiler chickens. To reduce the possible adverse effects of mycotoxin, zeolite (clinoptilolite) has used in poultry diets successfully (Oguz and Kurtoglu 2000). It acts as an adsorbent and reduces bioavailability of mycotoxin in gastrointestinal tract. The objective of this experiment was to use bakery wastes in broiler chicken rations and to study its effect on performance and role of zeolite in decreasing possible adverse effects of bakery wastes.

**Materials and methods.** 360,1-d-old, Ross male broiler chickens were randomly assigned to a completely randomized design experiment with 3×3 factorial arrangements with 4 replicates, 3 levels of bakery wastes (0, 10, 20%) at the expense of wheat and 3 levels of natural zeolite (0, 1, 2%) were used to evaluate the individual and combined effects of these factors. Isocaloric and isonitrogenous diets were formulated based on NRC 1994. The birds had 24-h access to feed, water and light. Bakery waste samples were analyzed for crude protein (CP), ether extract (EE), crude fiber (CF), nitrogen free extract (NFE), ash and sodium. Metabolizable energy was calculated from this equation:  $ME_n = 34.49CP + 76.1EE + 37.67NFE$  (NRC 1994). Performance data were recorded weekly. At 21, 42 and 49 d of the experiment one bird in each replicate was killed and its relative organ weights were measured. At 21 and 42 d, simultaneous with slaughter, blood sample from one bird in each replicate was collected and its serum was separated. Serum total protein, alanine aminotransferase, aspartate aminotransferase and lactate dehydrogenase activity were measured. Data were analyzed by using the GLM procedure of SAS (1996).

**Results** Mean feed intake and body weight (BW) gain data for the experiment are given in Tables 1 and 2, respectively. BW gains were significantly decreased when 20% bakery waste was added to the diet during 0-49 period ( $P < 0.05$ ). Zeolite didn't significantly change BW gain. The addition of 2% zeolite reduced feed intake in starter period ( $P < 0.05$ ). In spite of the reduction of feed intake, BW gain was not affected by 2% zeolite. 20% bakery waste decreased feed intake during finisher period ( $P < 0.05$ ). Addition of bakery waste or zeolite didn't alter feed conversion ratio. Zeolite didn't change any relative organ weights. Mortality was not affected by any dietary treatments. Bakery waste significantly increased relative bursa of fabricius weights ( $P < 0.05$ ). Addition of bakery waste didn't change serum total protein and serum lactate dehydrogenase, alanine aminotransferase and aspartate aminotransferase activity. There was no interaction between zeolite and bakery waste in all traits.

**Table 1** Mean bird feed intake in the experiment (g)

Day Factor	0-21	21-42	42-49	0-49
%Zeolite				
0	800.5 <sup>a</sup>	2597.1	1295.8	4693.4
1	821.6 <sup>a</sup>	2661.7	1372.6	4856.0
2	780.0 <sup>b</sup>	2607.3	1349.6	4736.9
P value	0.035	0.678	0.158	0.326
± SEM	10.657	55.270	28.018	77.780
%Bakery waste				
0	795.7	2599.2	1394.8 <sup>a</sup>	4789.6
10	793.2	2666.8	1342.7 <sup>a</sup>	4802.8
20	813.2	2600.1	1280.6 <sup>b</sup>	4693.9
P value	0.365	0.616	0.027	0.565
± SEM	10.657	55.270	28.018	77.78

**Table 2** Mean bird BW gain in the experiment (g)

Day Factor	0-21	21-42	42-49	0-49
%Zeolite				
0	445.0	1197.7	498.7	2141.4
1	447.3	1182.0	492.7	2122.0
2	438.7	1175.0	533.7	2147.4
P value	0.685	0.709	0.443	0.880
± SEM	7.204	19.760	24.182	37.039
%Bakery waste				
0	446.7	1214.5	539.6	2200.8 <sup>a</sup>
10	436.2	1184.2	501.8	2122.2 <sup>a</sup>
20	448.1	1156.0	483.6	2087.8 <sup>b</sup>
P value	0.453	0.132	0.266	0.106
± SEM	7.204	19.760	24.182	37.039

**Conclusions** According to the data replacement of 20% bakery waste reduced BW gain but inclusion of 10% didn't have any undesirable effect. The findings of this research suggest that 10% wheat can be replaced with bakery waste in the broiler chicken rations without any adverse effect on performance under the condition of this experiment.

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## Effect of dual feeding program and heat stress on broiler performance

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**Introduction** Heat stress is a major concern of the broiler industry due to the resulting decreased growth, feed intake and increased FCR ratio and mortality (Cooper and Washburn, 1998). Heat exposed birds decrease feed intake in order to reduce metabolic heat production and maintain homeothermy, resulting in slower growth. Genetic variation in responses to heat stress has been shown to exist between breeds (Yalcin, *et al.*, 1997). The dual feeding program may have transitory effects on heat production during the heat stress (De Basilio *et al.*, 2001). The aim of this research was to evaluate the response of broiler performance when a two different type of diet were offered during the heat stress.

**Materials and Methods** Eight hundred day old chick from two stocks (Arian and Rass) were used in a factorial design in which treatments were two types of feeding system namely dual feeding program and control (standard) and two ambient temperatures (36°C and 24 °C). Chicks were raised in normal condition until 21 days of ages and then they divided into two equal groups and reared either in heat stress or in normal ambient temperature. The main experimental period was four week. During the first week of study the in the stress group the temperature was raised by 2 °C daily and remained constant at 36 °C. Control or stressed group were assigned to diets either standard diet or dual feeding system. High protein fraction of dual feeding system was offered daily from 17.00 to 10.00 and high-energy fraction offered from 10.00 until 17.00 daily. Feed refusals were collected and weighed daily and the average daily individual feed intake determined for each week. Live weight was recorded weekly. Data were analysed by analysis of variance using general linear model (GLM: Statistical Analysis System Institute (SAS)).

**Results** During the first week of study due to gradual increase in temperature the effect of heat stress and broiler stock on FCR were not significant ( $P>0.05$ ) but effect of diet in this week was highly significant ( $P<0.01$ ). There was significant difference between heat stress and diets during second week of study ( $P<0.05$  and  $P<0.01$  respectively). However, during the third week of study just stock of bird had significant effect ( $P<0.05$ ).

**Table 1** Effect of heat stress, dual feeding program and stock on broiler FCR

Age	Ross				Arian				Level of Significance $\Phi$		
	Stress		Normal		Stress		Normal		Heat	Stock	Diet
22-28 day	1.79	1.65	1.86	1.60	1.85	1.66	1.73	1.53	ns	ns	**
29-35 day	1.56	1.35	1.44	1.18	1.48	1.54	1.60	1.23	*	ns	**
36-42 day	1.84	2.02	1.96	1.99	2.35	1.92	1.88	2.13	ns	*	ns

$\Phi$  \*  $P<0.05$ ; \*\* $P<0.01$ ; ns, not significant

**Conclusions** While there was no obvious significant interaction between stock and heat stress in all periods of study. However, trend were evident which suggested that genotypes selected by commercial breeders as superior under optimal condition may not maintain their superiority at high ambient temperature in hot regions or during the hot season especially for feed intake. The observations described here suggested that the effect of diet was highly significant during first two weeks of study. Although the best time for using dual feeding program is in high temperature but the behavioural variation in feed intake and different responses between individual and stock may account for the observed resistance of dual fed broiler to heat challenge.

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# The effect of severity and duration of early feed restriction on feed intake, body weight gain and feed conversion ratio of male broiler chickens

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**Introduction** One method of reducing feed cost is to restrict the feed in the early life of broilers. In this regard, Fontana *et al.* (1992) reported that early feed restriction programs in their experiments proportionally reduced the consumption of the starter diets by an average of 22% in restricted broilers when compared with controls. Energy restriction has also been shown to result in a reduction in metabolic energy loss leading to a reduced requirement for maintenance. If during refeeding, this low requirement is maintained and if growth resumed at a normal or above normal rate (compensatory growth), feed efficiency would be substantially improved, leading to an economical advantage. This experiment was conducted to determine the effect of severity of diet dilution (energy and protein) and duration of early feed restriction on feed intake (FI), body weight gain (BWG) and feed conversion ratio (FCR) of male broiler chickens.

**Materials and Methods** 504, one-day-old Ross male broiler chickens were used in a 2×4 factorial arrangement with completely randomized design and splitting the control in two parts. Factors of interest were: severity of diet dilution (Dil) with two levels of 25 and 50 percent ground rice hulls and duration of early feed restriction (Dur) were 4 periods of 0 (controls), 3, 6 and 9 days. It is notable that 25% diluted diet for 0 days (%25(0d)) and 50% diluted diet for 0 days (%50(0d)) are controls. Each of restricted and control treatments had 6 and 3 replicates, respectively. Each replicate had 12 day-old Ross male broiler chickens. All chicks were fed a standard starter diet as recommended by NRC (1994), from 1 to 7 day of age. They then were fed with their assigned treatments. As treatment application periods were finished, each experimental unit was fed with standard starter diet to 21 day of age and thereafter with grower and finisher diets until 42 and 56 day of age, respectively. Feed intake was recorded at 8, 11, 14, 17, 21, 28, 35, 42, 49 and 56 day of age. Data were analyzed by using the GLM procedure of SAS.

**Results** Mean feed intake and feed conversion ratio data for the experiment are given in table 1 and table 2, respectively. In the period of feed restriction (8-17 days of age), the feed consumption of restricted chickens was significantly less than controls ( $P<0.01$ ). Also, the consumption of starter diet in restricted chickens was significantly less than controls. Total feed consumption of chickens fed 25% diluted diet for 3, 6 and 9 days and chickens fed 50% diluted diet for 3 days was not significantly different from controls but total feed consumption of chickens fed 50% diluted diet for 6 and 9 days was significantly less than the control. In the period of feed restriction (8-17 days of age) and also in the period of 0-21 days of age, the BWG of restricted chickens were significantly less than controls ( $P<0.01$ ) but total BWG of restricted chickens was not significantly different from controls. In the period of feed restriction (8-17 days of age), the FCR of restricted chickens was significantly less than controls ( $P<0.01$ ). Also in the periods of 0-21 days of age, 21-42 days of age and 42-56 days of age, the FCR of restricted chickens were significantly less than controls ( $P<0.01$ ). Total FCR (0-56 days of age) of restricted chickens were significantly less than controls ( $P<0.01$ ).

Table 1. Mean feed intake of the chickens (g)

Age	8-17	0-21	0-56
Treatments			
%Dil(Dur)			
%25(0d)*	370.71	738.19	4989.80
%25(3d)	323.46	672.91	5025.70
%25(6d)	295.00	638.81	4905.80
%25(9d)	287.22	655.33	4905.80
%50(0d)*	384.10	747.79	5179.20
%50(3d)	290.72	635.88	4948.73
%50(6d)	239.91	568.47	4906.10
%50(9d)	163.17	500.63	4861.30
P value	0.0001	0.0001	0.2255
± SEM	10.206	18.141	85.518

Table 2. Mean feed conversion ratio of the chickens

Age	8-17	0-21	21-42	42-56	0-56
Treatments					
%Dil(Dur)					
%25(0d)*	1.81	1.75	2.33	2.93	2.39
%25(3d)	1.61	1.63	2.25	2.80	2.31
%25(6d)	1.65	1.66	2.22	2.78	2.30
%25(9d)	1.68	1.69	2.23	2.79	2.31
%50(0d)*	1.79	1.75	2.32	2.94	2.40
%50(3d)	1.72	1.71	2.22	2.77	2.31
%50(6d)	1.70	1.69	2.20	2.77	2.31
%50(9d)	1.71	1.68	2.19	2.76	2.31
P value	0.0001	0.0001	0.0001	0.0001	0.0001
± SEM	0.0122	0.0090	0.0088	0.0131	0.0109

\*Control treatments in table 1 and table 2.

**Conclusions** Because of FI and FCR in restricted chickens were significantly less than controls, it seems that early feed restriction in Ross male broiler chickens could lead to some economical benefits for broiler producers and according to the results of this experiment, the best severity and duration of early feed restriction is 50% diluted diet for 9 days.

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# The effect of severity and duration of early feed restriction on body weight and abdominal fat of male broiler chickens

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**Introduction** Early feed restriction programs designed for reducing body fat in broiler chickens rely on the phenomenon called compensatory growth to produce final body weight equal to controls and success of each feed restriction program is measured based on complete compensatory growth and the amount of body fat. Compensatory growth is defined as a recovery from a growth deficit resulting from a limited nutrient intake. Plavnik and co-workers in a series of studies showed that restricting metabolisable energy intake to providing only maintenance requirement for a short period early in the life of broilers resulted to a reduction in carcass and abdominal fats without affecting overall growth until 56 day of age. This means that there is potential to underfeed broilers for some time, without affecting weight at normal market age. The objective of this experiment was to determine the effect of severity of diet dilution (energy and protein) and duration of early feed restriction on body weight and abdominal fat of male broiler chickens.

**Materials and Methods** 504, one-day-old Ross male broiler chickens were used in a 2×4 factorial arrangement with completely randomized design and splitting the control in two parts. Factors of interest were: severity of diet dilution (Dil) with two levels of 25 and 50 percent ground rice hulls and duration of early feed restriction (Dur) were 4 periods of 0 (controls), 3, 6 and 9 days. It is notable that 25% diluted diet for 0 days (%25(0d)) and 50% diluted diet for 0 days (%50(0d)) are controls. Each of restricted and control treatments had 6 and 3 replicates, respectively. Each replicate had 12 day-old Ross male broiler chickens. All chicks were fed a standard starter diet as recommended by NRC (1994), from 1 to 7 day of age. They then were fed with their assigned treatments. As treatment application periods were finished, each experimental unit was fed with standard starter diet to 21 day of age and thereafter with grower and finisher diets until 42 and 56 day of age, respectively. Body weights (BW) were recorded at 11, 14, 17, 21, 42 and 56 day of age. At the end of experiment, one bird from each replicate was killed and its abdominal fat (AF) including fats around gizzard and cloaca was collected and weighed. Data were analyzed by using the GLM procedure of SAS.

**Results** Mean BW and AF data of the chickens are given in tables 1 and 2, respectively. At the 11d of age, the BW of restricted chickens was significantly less than controls ( $P<0.01$ ). At the 14 and also 17d of age, the BW of chickens fed 25% diluted diet for 3 days were not significantly different from controls, but the BW of chickens fed 25% diluted diet for 6 and 9 days and the BW of chickens fed 50% diluted diet for 3, 6 and 9 days were significantly less than controls. At the 42 and also 56d of age, the BW of restricted chickens was not significantly different from controls. The weight of AF (AFW) and also the percent of AF (AFP) in restricted chickens were significantly less than controls ( $P<0.01$ ).

Table 1. Mean body weight of the chickens (g)

Age	11	14	17	42	56
Treatments					
%Dil(Dur)					
%25(0d)*	147.08	217.50	308.19	1494.44	2121.82
%25(3d)	140.24	215.84	305.87	1535.71	2216.32
%25(6d)	138.68	194.72	280.14	1477.50	2166.53
%25(9d)	138.75	198.51	274.10	1479.77	2160.36
%50(0d)*	147.22	223.29	315.68	1512.59	2192.89
%50(3d)	117.65	191.46	271.88	1479.98	2179.73
%50(6d)	114.72	149.90	242.98	1453.68	2159.01
%50(9d)	112.65	153.58	197.02	1406.07	2137.06
P value	0.0001	0.0001	0.0001	0.1540	0.6261
± SEM	2.2740	4.2323	6.8200	35.6750	37.3828

\* Control treatments in table 1 and table 2.

Table 2. The AFW and AFP of the chickens

Age	AFW	AFP
Treatments	(g)	
%Dil(Dur)		
%25(0d)*	61.23	3.24
%25(3d)	54.18	2.66
%25(6d)	43.80	2.20
%25(9d)	45.17	2.35
%50(0d)*	69.13	3.58
%50(3d)	54.43	2.74
%50(6d)	47.02	2.35
%50(9d)	47.33	2.51
P value	0.0019	0.0001
± SEM	4.2141	0.1750

**Conclusions** According to data, compensatory growth was achieved in restricted chickens and early feed restriction caused reduction in AFW and AFP in restricted chickens when compared with controls. Therefore it seems that early feed restriction in Ross male broiler chickens could lead to some economical benefits for broiler producers and according to the results of this experiment, the best severity and duration of early feed restriction is 50% diluted diet for 9 days.

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## Egg selenium concentrations in breeder hens fed Na-selenite or Sel-Plex® supplemented diets

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**Introduction** Feed grains available in Britain contain low concentrations of natural selenium. As the grains in most cases do not cover the requirements of the animals, selenium supplementation of diets is a standard practice in feed manufacturing. Selenium supply to the breeder hens impacts the selenium content of the egg, the developing embryo and therefore the antioxidant status of the chick at hatch (Surai, 2000). The aim of this study was to determine the effect of selenium from Sel-Plex® (Alltech Inc.) on the transfer of selenium into the egg of broiler breeder hens under commercial conditions.

**Materials and Methods** The trial included a total of 18 commercial breeder flocks (Ross 308 and Cobb 500). The trial was setup as a randomized block design with 9 blocks (farms) and 2 houses per farm. All flocks were fed diets meeting commercial nutrient specifications. Prior to the trial all diets were supplemented with 0.2 ppm of selenium from Na-selenite. During the trial, diets were either supplemented with 0.2 ppm of selenium from Na-selenite or from Selplex® (200g/tonne). Sel-Plex is an organic selenium supplement produced from sodium selenite and yeast. After a minimum of 3 weeks supplementation (adaptation period) 12 eggs per flock were randomly selected, separated into shell, yolk and albumen and the fractions were pooled and weighed. The concentration of selenium in both the yolk and albumen were analyzed. Selenium was analyzed using hydride generation atomic absorption spectrometry after hydrolysis of the sample with a mixture of nitric/perchloric/sulfuric acid. Data were analyzed as a randomized block design by ANOVA.

**Results** The supplementation with Selplex® led to a significant increase in selenium concentrations of both the yolk and the albumen compared to the Na-selenite treatment. Selenium content of the total egg was 17.43 and 21.30 µg for the Na-selenite and the Selplex® treatment, respectively. These values are in the same range as values (14 and 22 µg) reported by Paton *et al.* (2000) who were feeding diets with similar selenium supplementation to layers. The increase in selenium concentration with Selplex® for the yolk, the albumen and the total egg was 13%, 65% and 22%, respectively.

**Table 1** Selenium content of yolk, albumen and total egg from hens fed Na-selenite or Sel-Plex®

Treatment	Yolk		Albumen		Egg
	ng/g	µg (total)	ng/g	µg (total)	µg (total)
Selenite	595.1	13.2	125.3	4.25	17.43
Sel-Plex®	638.3	14.3	206.6	7.00	21.30
SE	5.93	0.18	3.92	0.13	0.20
P	< 0.001	< 0.005	< 0.001	< 0.001	< 0.001

### Conclusions

Substitution of inorganic selenium with Sel-Plex® significantly increased egg selenium content. Significant increases were seen in both the yolk and albumen fractions of the egg.

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## Performance and carcass measures of broilers maintained on diets containing Biomin (a symbiotic natural growth promoter) under tropical conditions

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**Introduction** The need to provide protein at a low cost to consumers in Nigeria, has stimulated continued search for more suitable combination of known nutrients, and for new additives which will increase the efficiency and growth rate and therefore level of production of poultry birds. These widespread efforts have led to the present use of growth promoters (variously called growth permitters, growth enhancers, biostimulators). Growth promoters are antimicrobials, synthetic agents or mixtures of these. They directly or indirectly enhance digestion and hence anabolism. They remove pathological conditions and therefore cause growth promotion. This study reports the use of one of such commercial growth promoters recently introduced into the poultry market in Nigeria on the performance of broilers

**Materials and methods** Eighty (80) day old broiler chicks were allocated to two experimental diets in a completely randomized design on body weight basis. At the starter and finisher phases there were 40 birds per treatment shared into 4 replicates with 10 birds each. Diet 1 was a control standard diet with no Biomin (a symbiotic natural growth promoter based on natural raw materials combining the beneficial effects of probiotics, prebiotics and immune-stimulating substances) while Diet 2 had Biomin added to it at the recommended rate of 100 g per 100 kg of diet. At the end of 28 days, the diets of the birds were changed to finisher diets with Diet 2 containing the same level of Biomin. The birds were subjected to standard routine broiler management. The finisher phase ended after additional 28 days. Body weight gain, feed intake, feed conversion ratio and carcass measures were estimated and statistically analyzed (SAS Institute, 1995) and significant between means separated by Duncan's multiple range test (Duncan, 1955).

**Results** Feed consumption, body weight gain, feed conversion ratio and metabolizable energy intake were significantly improved ( $P<0.05$ ) with addition of Biomin to the diet both at starter and finisher phases (Table 1). The packed cell volume, red and white blood cells and plasma globulin, increased though not significantly with addition of Biomin to the diet. Plasma total protein, albumin, eviscerated weight, breast, drumstick and heart were significantly ( $P<0.05$ ) increased by the Biomin-based diet (Table 2).

**Table 1** Effect of Biomin on performance of broilers

	Diet 1 (Control)	Diet 2 (+ Biomin)
<b>Parameters</b>		
Average daily feed intake (g)	76.18±0.93a	95.77±0.68b
Average daily weight gain (g)	34.09±0.23a	44.82±0.87b
Feed conversion ratio	2.23±0.21a	2.14±0.25b
Metabolizable energy intake (KJ)	957.60±19.88a	1206.66±21.33b

Note: Means on the same with different superscripts are significantly ( $P<0.05$ ) different.

**Table 2** Effect Biomin on haematological values, carcass measures and organ weights

	Diet 1 (Control)	Diet 2 (+ Biomin)
<b>Parameters</b>		
Packed cell volume (%)	23.00±0.58	27.00±0.60
Red blood cells ( $\times 10^6 \text{ ul}^{-1}$ )	3.92±0.05	4.19±0.06
White blood cells ( $\text{ul}^{-1}$ )	9.93±0.30	10.83±0.44
Plasma total protein (g /100ml)	4.97±0.22a	6.00±0.06b
Plasma albumin (g /100ml)	1.77±0.03a	2.43±0.18b
Plasma globulin (g/100ml)	3.20±0.21	3.70±0.06
Breast (g)	260.00a	310.00b
Eviscerated weight (g)	990.00a	1210.00b
Drumstick (g)	146.00a	193.00b
Heart (g)	10.83a	15.00b

Note: means on the same with different superscripts are significantly ( $P<0.05$ ) different.

**Conclusion** Results of the study showed that addition of Biomin resulted in improved performance and better carcass in broilers under tropical conditions

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## Steroid hormones concentration of the preovulatory ovarian follicles of the goose

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**Introduction** Ovarian follicles are the most important steroids producing structures of the avian ovary. The ovary of a mature goose generally contains 7-9 large preovulatory follicles arranged in a follicular hierarchy, several postovulatory follicles, and numerous small follicles, which have not entered the follicular hierarchy. It has been reported that in avian preovulatory follicles biosynthesis of sex steroid changes during maturation (Gomez et al., 1998; Lee et al., 1998). The cell theory for steroid production suggested that granular layer of preovulatory follicles primarily produce progesterone that are required as substrate for the production of androgen and estradiol by theca layer (Huang et al., 1979). The recently multiple-cell theory of steroidogenic suggest that theca layer can also synthesize progesterone, androgen and estradiol independent of granulosa layer (Nitta et al., 1991).

Despite strong interest in bird reproductive system there is little information on the goose ovarian system. Therefore the aim of the study was to determine concentration of progesterone, estradiol and androgens in different sizes of the preovulatory follicles.

**Materials and Methods** The concentrations of steroid hormones (P4 – progesterone, E2 – estradiol and A – androgens) were determined in isolated theca and granular layer of the three largest preovulatory ovarian follicles of the goose. The studies were carried out on 12 one-year-old Zatorska geese during the reproductive cycle. Preovulatory follicles (F1, F2 and F3) which had entered the hierarchy were isolated from ovaries approximately after egg was layed and before the ovulation. Yolk was removed from the tissues and were homogenized. Steroid hormones were measured by RIA methods (Spectria Orion Diagnostic, Finland).

The concentrations of steroid were computed in pg or ng/mg protein and were expressed as means  $\pm$  SD. Statistical differences were calculated using Student's *t*-test and Duncan's new test.

**Results** Mean steroid concentration data for the study are given in Table 1. The major findings of the present study were, that in preovulatory follicles of the goose; 1.) maximum level P<sub>4</sub> was recorded in granular layer and the concentration P<sub>4</sub> significant increased as follicles matured. In all examined follicles level P<sub>4</sub> in the theca layer was significantly lower than the granular layer; 2.) the greatest E<sub>2</sub> concentration was observed in the theca layer, and next level E<sub>2</sub> was decreased significant with follicular size. In the granular layer the E<sub>2</sub> concentration was very low and remained constant during the third and second position in the hierarchy, and declined throughout the first position; 3.) changes in concentrations in the theca and granular layer were similar. There was a gradual decrease in both layers during follicular maturation.

**Table 1** Steroid concentration P<sub>4</sub> (ng/mg protein  $\pm$  SD), E<sub>2</sub> and A (pg/mg protein  $\pm$  SD) in the three largest preovulatory follicles (F1, F2, F3) of the goose.

	Progesterone [ng]		Estradiol [pg]		Androgens [pg]	
	Granular layer	Theca	Granular layer	Theca	Granular layer	Theca
<b>F1</b>	68.7 $\pm$ 16.3 <sup>c</sup>	2.61 $\pm$ 0.24 <sup>c</sup>	bs	19.1 $\pm$ 2.7 <sup>d</sup>	116.2 $\pm$ 9.7 <sup>c</sup>	68.2 $\pm$ 7.4 <sup>f</sup>
<b>F2</b>	27.2 $\pm$ 6.4 <sup>b</sup>	1.73 $\pm$ 0.21 <sup>d</sup>	7.8 $\pm$ 0.9 <sup>a</sup>	91.3 $\pm$ 10.8 <sup>c</sup>	256.2 $\pm$ 23.9 <sup>ab</sup>	127.9 $\pm$ 19.1 <sup>ce</sup>
<b>F3</b>	11.7 $\pm$ 0.7 <sup>a</sup>	1.56 $\pm$ 0.18 <sup>d</sup>	9.1 $\pm$ 0.7 <sup>a</sup>	124.8 $\pm$ 16.5 <sup>b</sup>	302.1 $\pm$ 26.8 <sup>a</sup>	198.2 $\pm$ 16.5 <sup>d</sup>

a, b, c, d, e f - significant  $p < 0,05$

bs – below sensitivity

**Conclusions** It was concluded that, similar to hen, granular layer of the preovulatory follicles of the goose is the major source of progesterone while theca layer is the principle source of estradiol. Additionally, in the granular layer steroidogenic activity dramatic increase while in the theca layer gradual decrease as the follicle approaches ovulation.

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## Effect of different levels of bacterial probiotic on broilers performance

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**Introduction** The microbial populations in the gastrointestinal tracts of poultry play a key role in normal digestive processes and in maintaining animal health. Disease- and stress-induced changes in the physicochemical environment in the gastrointestinal tract, or simple changes in feed management practices can significantly influence the microbial populations and their effects on animal performance and health. In the last five decades, increased knowledge of the factors that influence the activities of microorganisms in the alimentary tract has helped to define the critical role of these symbiotic organisms. Probiotics, competitive exclusion and direct-fed microbial feed supplements can be used as a strategic tool for managing these microbial populations. The aim of this trial was study of effect of different levels of bacterial probiotic on broilers performance and some of blood factors.

**Materials and methods** This experiment was conducted in a randomized complete block design (RCBD) and included 600 Ross broiler chicks (male and female) which were divided into four groups with five replicates. This experiment was conducted in two periods starter 0-21, and grower 22-42, days. All of the diets were isocaloric and isonitrogenous. In this experiment, the birds received 0, 800, 1000, 1200 gr probiotic (*Bacillus subtilis* and *Bacillus licheniformis*) / ton diets in the starter period, and 0, 320, 400, 480 gr probiotic / ton diets in the grower period, which were termed T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub>, respectively. The used probiotic (BioPlus 2B) was a powder in which the active ingredient is an equal mixture of spray-dried spore-forming bacteria, *Bacillus licheniformis* and *B. subtilis*, at a minimum concentration of  $3.2 \times 10^9$  viable spores/g. Our measurements were, weight gain, feed intake, feed efficiency, mortality, carcass quality, serum cholesterol, blood hemoglobin and number of white blood cells.

**Results** Weight gain at starter period was significantly affected by dietary treatments ( $P < 0.05$ ), but weight gain at grower period was not affected by using probiotic supplement ( $P > 0.05$ ). At the end of trial, the T<sub>4</sub> had maximum weight gain, and T<sub>1</sub> had minimum weight gain. The feed intake and mortality were not affected by experimental diets ( $P > 0.05$ ). Analysis of variance showed no significant difference between treatments for feed efficiency ( $P > 0.05$ ), but there was significant difference between mean of treatments that were derived from Duncan's multiple range test ( $P < 0.05$ ). There were no significant difference between groups in dressing, breast meat and thigh percent, at the end of 6<sup>th</sup> week ( $P > 0.05$ ). The first treatment had the highest abdominal fat (3.04 percent) and fourth treatment had the lowest abdominal fat (2.43 percent), but among them, there was no significant difference ( $P > 0.05$ ). Analysis of variance showed no significant difference between treatments for serum cholesterol content ( $P > 0.05$ ), but significant difference between mean of treatments were derived from Duncan's multiple range test ( $P < 0.05$ ). The blood hemoglobin content was not affected by experimental diets ( $P > 0.05$ ). The number of white blood cells were significantly affected by dietary treatments ( $P < 0.05$ ), the T<sub>4</sub> had maximum number of white blood cells, and T<sub>1</sub> had minimum number of white blood cells.

Measurments	Unite	Treatments			
		1	2	3	4
Weight gain (0-21)	(gr)	427.52 <sup>b</sup> ±11.013	436.32 <sup>ab</sup> ±10.356	451.92 <sup>a</sup> ±10.697	453.86 <sup>a</sup> ±19.703
Weight gain (22-42)	(gr)	1141.49±23.488	1148.27±19.446	1168.48±69.493	1174.64±70.036
Weight gain (0-42)	(gr)	1569.01±29.056	1584.59±25.445	1620.40±66.159	1628.50±52.566
FCR (0-21)	(gr/gr)	1.83 <sup>a</sup> ±0.0323	1.74 <sup>b</sup> ±0.0131	1.72 <sup>b</sup> ±0.0337	1.72 <sup>b</sup> ±0.0912
FCR (22-42)	(gr/gr)	2.09±0.0738	2.05±0.0136	2.04±0.1027	2.04±0.1008
FCR (0-42)	(gr/gr)	2.02 <sup>a</sup> ±0.0505	1.96 <sup>ab</sup> ±0.0072	1.95 <sup>ab</sup> ±0.0770	1.94 <sup>b</sup> ±0.0548
Dressing	(%)	68.497±1.486	68.383±3.151	70.166±2.302	70.302±3.084
Breast meat	(%)	30.227±2.375	29.500±2.878	29.544±2.100	30.197±1.691
Thigh	(%)	30.182±1.194	30.999±2.009	30.394±1.302	30.659±1.260
Abdominal fat	(%)	3.040±0.249	2.598±1.007	2.764±0.309	2.431±0.455
Serum cholestrol content	(mg/dl)	129.62 <sup>a</sup> ±17.752	125.85 <sup>ab</sup> ±12.745	117.34 <sup>ab</sup> ±11.367	115.51 <sup>b</sup> ±11.900
Blood hemoglobin content	(g/dl)	13.33±1.135	13.44±0.911	13.21±0.597	13.49±0.706
White blood cells	(n/mm <sup>3</sup> )	24280 <sup>b</sup> ±3746.94	27500 <sup>ab</sup> ±5142.42	26900 <sup>ab</sup> ±5591.66	30950 <sup>a</sup> ±3911.88

a, b means without a common letter differ at  $P < 0.05$

**Conclusions** The results from this study show that supplementation of bacterial probiotic (*Bacillus subtilis* and *Bacillus licheniformis*) in broiler feed not only caused improvement in performance, but also decreased serum cholesterol content and stimulated the immune system of broilers.

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# Effect of CRYSTALYX® on the performance of breeding ewes during first three months of pregnancy while grazing grass outdoors

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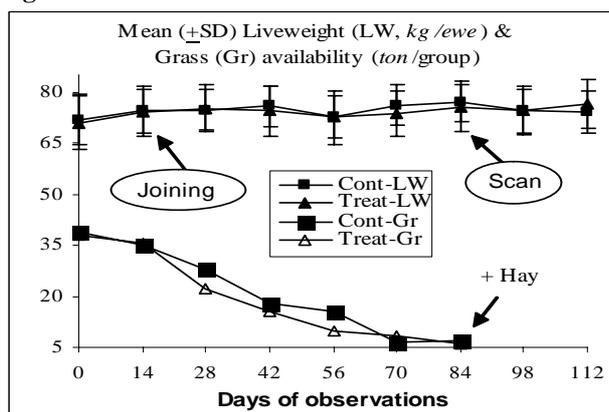
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**Introduction** It is recognised that feeding grass alone cannot satisfy the nutritional needs of breeding sheep and this consequently can impair their health and production. However, if grass is fed together with a suitable supplement that is specifically formulated to compensate for the nutrient deficiency of that grass then its utilisation followed by animal performance can be improved. This study examined the effect of feeding *ad libitum* an Extra High Energy (16MJ ME/kg DM) supplement (CRYSTALYX®) containing sugars, oil, protein (12%) and minerals on the performance of breeding ewes when grazing grass outdoor pre-joining and during first 3 months of post-joining with rams.

**Materials and methods** Around 160 Mule ewes with initial mean live-weight (LW) of 66±8kg (±SD) were divided in mid October 2001 into Control and Treatment groups, balanced for age, LW and past lambing records at the University Farm. Four Texel rams with initial mean (±SD) LW of 96±4 kg were also divided into Treatment and Control groups according to their breeding history. Initially, each group of ewes and rams was flushed on a separate field containing similar type and almost equal amount of perennial ryegrass. Only one mini tub containing 22.5kg CRYSTALYX® for Treatment rams and four such tubs for Treatment ewes were evenly placed in the respective fields. The Treatment rams were given *ad libitum* access to the tub a month in advance than the Treatment ewes which started consuming CRYSTALYX® about 2 weeks before the Treatment rams were joined in on 2<sup>nd</sup> November 2001. On the same day, the Control rams were also joined in with the Control ewes. Grass samples were collected by using a motorised shearer from 10 randomly chosen 0.5m quadrants per field at the start of flushing and then fortnightly to estimate the quality and quantity of available grass. The tubs were checked regularly and replaced if empty with new ones. The fortnightly intakes of CRYSTALYX® by the Treatment ewes were then calculated. LW of all ewes and rams were recorded every fortnight. The ewes were checked for cover during the first month post-joining and then scanned for pregnancy in January 2002, over two months post-joining. All data were statistically analysed to study the effect of CRYSTALYX®, confounded with field, on sheep growth and breeding performance. Significance was declared when P<0.05.

**Figure1**



**Table1** Various parameters of sheep performance

Parameters	Control n=82	Treatment n=80	SE
Condition Score at Joining	3.7	3.5	0.067
3 months later	2.5	2.8	0.056
Crystalyx intake (g/ewe/day) at Joining	nil	49	-
3 months later	nil	90	-
Mean daily gain (g/ewe)	19	25	3.45
Cover within 17 days of joining (%)	92	96	-
Expected lamb number Per scanned ewe	2.22	2.23	0.074

**Results** Figure 1 shows that both groups of ewes were able to maintain their live-weights (LW) despite continuous decline in the grass availability in both fields during this study. It appeared that adequate quantity of grass was perhaps available to each group of ewes in each field to maintain their LW at most times. However, the Treatment ewes, on average (SE 3.45, P>0.05), grew (g/ewe/day) faster (25) than the Control ewes (19) (Table 1) even when there was a greater decline in grass availability over time in Treatment group. This faster growth rate may have been due to the consumption of essential nutrients from CRYSTALYX® (Table 1) which was shown to improve *in vitro* digestion of forages (Chaudhry et al 2002). The improved digestion could have enhanced the utilisation of grass followed by growth in Treatment ewes. The Treatment ewes tended to maintain a better condition score than the Control ewes (Table 1) after three months of joining. More ewes were covered in Treatment group by the rams within the first 17 days of joining than those in the Control group. Although the scanning data did not show any difference between Treatment and Control groups for the expected lamb numbers /ewe, the expected lamb number was very high for both groups.

**Conclusion** The scanning data for expected lambs /ewe did not differ between Control and Treatment groups. However, it appeared that the Treatment ewes consuming CRYSTALYX® tended to grow faster, had a better condition score and showed greater service cover than their Control counterparts. Further studies using the actual lambing data are continued to observe the effect of feeding CRYSTALYX® on other breeding parameters of these ewes.

**Acknowledgements** Thanks to Jim Wightman, David Routledge and other Farm staff for their assistance.

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# Effects of time spent on pasture and the characteristics of the concentrate on milk yield, milk composition and body reserves in the Latxa dairy ewe

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**Introduction** In the basque country, in the spring, dairy ewes can spend a limited number of hours between 4 and 8 hours on pasture (Oregui et al., 1997). Besides grazing, ewes receive a supplementation of concentrates and forages in parlour. Therefore the characteristics of this supplementation are very important. In this sense, there is evidence that an increase in rumen undegradable protein could lead to an increase in milk production (Santos et al., 1998). In recent years, it has been shown the potential of tannins to decrease protein degradation in the rumen (Hervas, 2001). However, there is a lack of knowledge concerning the effect of increasing the content of rumen undegradable protein of the concentrate in a production system like the one in the basque country.

**Materials and methods** Two concentrates differing in the soluble tannin (quebracho) content were formulated. *In situ* protein degradability was determined on a composite sample of each concentrate. A total of 48 multiparous Latxa dairy ewes on average 42 days in milking at the beginning of the study, were grouped into 4 blocks of 12 ewes on the basis of milk yield, protein content, fat content, body weight and body conditioning score so that blocks were homogeneous. Blocks were randomly assigned to one of the following experimental concentrates: 1) Concentrate containing 90 g/kg DM soluble tannins (T) or 2) soluble tannin free concentrate (C). 520 g DM of T and 484 g DM of C were offered in two equal feedings during milking times so that rations were isoenergetic and isoproteic. The diets were fed for 5 weeks. Ewes on both experiments were on pasture either 4 hours (4H), from 9:00 to 13:00 resulting in 4HT and 4HC, or 7 hours (7H), from 9:00 to 16:00 resulting in 7HT and 7HP. When not on pasture ewes were housed in a free-stall barn and were fed 271 g DM of alfalfa after the evening milking. Each block of ewes was allocated to a different paddock. Sward heights were measured twice a week with a hand stick. Paddock surface was managed to keep the sward height between 6 and 8 cm. Ewes were milked twice daily. Milk yield for each ewe was recorded and collected once a week at morning and evening milking. Milk samples were analysed for protein and fat contents. Body weight and body condition score for each ewe was recorded weekly. Data were analysed using the GLM procedures with concentrate type, time on pasture, week of experience and all possible interactions as fixed factors and initial values as covariates.

**Results** In terms of protein degradability, the addition of tannins to the concentrate did not cause a significant reduction neither in the soluble fraction degradability (0.17 vs 0.20  $P < 0.14$ ) nor in the degradable fraction (0.80 vs 0.78  $P < 0.18$ ) but there was a trend for the rate of degradation (0.097 vs 0.057  $P = 0.06$ ) for C and T respectively. As a consequence, there were significant differences in the effective degradability of the protein considering  $K_p = 6\%h^{-1}$  (0.66 vs 0.58  $P < 0.05$ ) for C and T respectively. The addition of tannins to the concentrate did not have a significant effect on milk yield, milk protein content, standard milk production or body condition score. On the other hand, it significantly decreased milk fat content and significantly increased body weight (Table 1). When considering the time spent on pasture, the ewes that spent 7 hours significantly increased milk yield, milk protein content, standard milk production and body weight but they had a significant decrease in milk fat content (Table 1).

**Table 1** Effects of protein degradability and time on pasture on lactational performance and body reserves evolution

	concentrate		time		sem	Statistical significance		
	C	T	4H	7H		concentrate	time	concentrate*time
Milk yield (ml/d)	1259	1298	1222	1334	277.1	ns	***	ns
protein (%)	5.1	5.19	5.09	5.21	0.278	ns	*	ns
fat (%)	6.60	6.36	6.75	6.20	0.585	*	***	ns
Standard Milk Production (ml/d)	1142	1140	1105	1176	145.8	ns	**	ns
Body weight (Kg)	58.5	59.0	57.8	59.8	1.66	**	***	ns
Body condition score	2.70	2.69	2.67	2.72	0.314	ns	ns	ns

Significantly different, \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$

**Conclusions** This study has indicated that increasing the tannin content of the concentrate significantly reduced protein degradability but it did not improve lactational performance in the Latxa dairy ewe production system. On the other hand, in this study it has been observed that time spent on pasture, involving 21% more grazing time (data not shown), has positive but limited effects on lactational performance.

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## Intake and apparent digestibility of *Leucaena leucocephala* for Santa Inês sheep diet

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**Introduction** The use of leguminous forages is an alternative of protein supplementation in animal diets. *Leucaena leucocephala* (leucaena) is lifelong leguminous forage that can be directly grazed or harvested, offered fresh, hay or silage to animals. Many leguminous show anti-nutritional factors that may reduce the use of these plants in animal diets. Condensed tannin (CT) is one common anti-nutritional factor present in the leucaena. The objective of this work was to evaluate the use of *Leucaena leucocephala* in Santa Inês sheep diets on intake and digestibility parameters.

**Material and Methods** Twelve Santa Inês wether, average body weight (BW) of 35 kg, were allocated to six mixed diets in a change-over design in three periods of twenty-one days. Diets were based on *Cynodon sp* and supplemented with 20 (L20), 40 (L40) and 60% (L60) of leucaena hay. Control diets CL20, CL40 and CL60 were also based on *Cynodon sp* supplemented with a concentrate containing soybean meal (49.24 %), citrus pulp (49.24 %) and urea (1.5 %) in order to offer approximated acid detergent soluble protein (ADSP) rate as diets L20, L40 and L60, respectively. During each period animals were kept in individual pens for a seven-day feed adaptation period followed by a seven-day intake trial where voluntary dry matter intake (VDMI) was estimated. The lambs were then moved into metabolic cages indoors for a five-day digestibility trial estimating apparent total digestibility of dry matter (DMD), crude protein (CPD), neutral detergent fibre (NDFD) and of acid detergent fibre (ADFD). VDMI was estimated based on total offered minus refusals, allowing refusals between 10 – 20%. In both trials, animals had free access to water and mineral lick. Diets were offered at 8:30 h and 16:00 h. Chemical composition was based on AOAC (1995) and Van Soest and Wine (1967). The determination of CT followed HCL-Butanol methodology (Makkar, 2000). Non-orthogonal contrasts were employed to compare the animal responses among leucaena and control treatments. Linear and square regressions were used to analyse the difference between levels of leucaena included (SAS, 2000).

**Results** *Voluntary dry matter intake trial:* voluntary dry matter intake (VDMI) was estimated among diets and the results obtained are presented on Table 1. Despite the presence of tannins (CT) in leucaena diets, VDMI was not affected between leucaena and control diets ( $P>0.05$ ) averaging 62 and 57 g kg<sup>-0.75</sup>BWd<sup>-1</sup>, respectively.

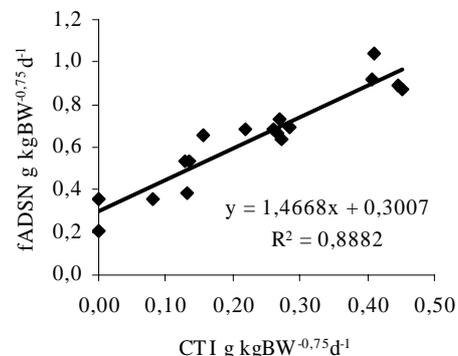
This suggests that either the quantity or the type of CT in the leucaena diets did not affect the acceptability. Comparing levels of leucaena included, leucaena fed at 40 and 60 % (L40 and L60) had higher VDMI than diet L20 did ( $P<0.05$ ).

*Digestibility trial:* DMD, CPD and ADFD (Table 1) showed lower values than control diets did ( $P<0.05$ ). Leucaena diets presented higher acid detergent lignin (ADL) than control diets averaging 13 % and 8 %, respectively. Faecal ADSP of leucaena diets was higher than control ( $P<0.05$ ) and increased according to CT increments (Figure 1).

**Table 1** Voluntary dry matter intake (VDMI)<sup>1</sup>, dry matter intake (DMI) condensed tannin intake (CTI), digestibility of dry matter (DMD), crude protein (CPD) and acid detergent fibre (ADFD) and faecal acid detergent soluble nitrogen (fADSN) in Santa Inês sheep

	DIETS <sup>2</sup>						SED <sup>4</sup>	CV <sup>5</sup>
	L20*	L40*	L60*	CL20*	CL40*	CL60*		
VDMI <sup>1</sup> g kgBW <sup>-0.75</sup> d	52 <sup>b</sup>	68 <sup>a</sup>	62 <sup>ab</sup>	58 <sup>ab</sup>	54 <sup>b</sup>	60 <sup>ab</sup>	3.6	15
DMI g kgBW <sup>-0.75</sup> d <sup>-1</sup>	57 <sup>b</sup>	69 <sup>a</sup>	70 <sup>a</sup>	62 <sup>ab</sup>	58 <sup>b</sup>	68 <sup>a</sup>	2.9	11
CTI g kgBW <sup>-0.75</sup> d <sup>-1</sup>	0.07	0.25	0.36	0.01	0.01	0.01	0.02	52
DMD g kg <sup>-1</sup> DM	390 <sup>a</sup>	374 <sup>a</sup>	390 <sup>a</sup>	511 <sup>b</sup>	504 <sup>b</sup>	528 <sup>b</sup>	2.1	11
CPD g kg <sup>-1</sup>	337 <sup>c</sup>	320 <sup>c</sup>	367 <sup>c</sup>	532 <sup>b</sup>	630 <sup>a</sup>	667 <sup>a</sup>	3.0	15
ADFD g kg <sup>-1</sup>	348 <sup>b</sup>	269 <sup>b</sup>	251 <sup>b</sup>	470 <sup>a</sup>	557 <sup>a</sup>	541	3.9	23
fADSN <sup>3</sup> g kgBW <sup>-0.75</sup> d <sup>-1</sup>	0.40	0.68	0.84	0.29	0.31	0.42	0.03	17

<sup>1</sup>intake trial; <sup>2</sup>L20 = 20 % leucaena; L40 = 40 % leucaena; L60 = 60% leucaena; CL20 = control L20; CL40 = control L40; CL60 = control L60; <sup>3</sup> = fecal N – fecal ADIN; <sup>4</sup>standart error deviation; <sup>5</sup>coefficient of variation \* means followed by a different superscripts in row differ significantly ( $P<0.05$ )



**Figure 1** Linear regression between condensed tannin intake (CTI) and faecal ADSP

**Conclusion** Inclusion of 40% of *Leucaena leucocephala* increased VDMI. The low DMD and CPD for leucaena diets were due to high ADL present. Despite the low level of CTI, the higher ADSP values suggest an additive action of ADL and CTI. More studies on origin of faecal soluble N and also reactivity of tannin have to be performed for better conclusions

**Acknowledgements** This experiment was part of project supported by FAPESP.

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## The effects of nutrition and age on characteristics of fibers Raeini Cashmere goat

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**Introduction** Traditionally goats are indeed important in Iran especially for milk and fibers. This country has nearly 5000000 cashmere producing goat, which produce approximately 1500 metric ton of raw cashmere. So research on environmental factors which have effect on cashmere production, is very vital to economy of the country. Raeini breed is the most productive Cashmere goat in Iran. However, There are no detailed descriptions of the effects of nutrition on cashmere characteristics in Raeini goat. There is some debate on the effects of nutrition on cashmere growth. In some studies cashmere growth does not respond to increased feed intake above maintenance. While some reviewers have concluded that nutrition does influence cashmere growth (McGregor, 1998). The objective of this study was to describe the effects of feeding level on the cashmere growth of Raeini female goats at two different ages.

**Materials and methods** Twelve 6-month old (young) and twelve 18-month old female (old) Raeini goats were selected for use in the experiment. The trial was conducted at the Khalat-Pushan Research Station, University of Tabriz. Initial live weight ( $\pm$  standard errors) for the young and old goats was  $16.42 \pm 1.22$  and  $29.54 \pm 4.33$  kg respectively. The animals were individually penned and fed on commercial Lucerne for 6 months (+ 2 months pre-experiment period). All goats do not permitted to mate, to avoid influence of pregnancy and lactation on cashmere growth. Maintenance requirements and near *ad libitum* levels of feed intake were estimated from the studies of Kloren et al. (1993). Four 6-month-old and four 18-month-old female goats were randomly assigned to maintenance (M), 0.7 M, 1.4 M and 1.8 M feeding groups in a 2 (age) x 4 (level) factorial experiment. Feed offered was adjusted every 2 weeks to correct for changes in live weight. Patches of fleece from defined areas were repeatedly shorn at 4 weekly intervals from the right mid-side of each goat. For follicle study, skin biopsy was taken monthly from left mid-side.

**Results** Cashmere diameters of goats during the experiment period are given in Table 1. There were no significant effect of feeding levels or age on cashmere and hair weights, secondary follicle activity, cashmere length and yield. Fleece and live weight for young goats were significantly ( $P < 0.01$ ) lower than those of old goats. The goats fed M, 1.4M and 1.8M had high diameter of cashmere than those fed 0.7M and the difference was significant ( $P < 0.05$ ). There was no significant difference ( $P > 0.05$ ) between old and young goats in diameter of cashmere during the experiment period. Diameter of cashmere during different months was variable. The mean of fiber growth rates was maximum in June and July (1.64 and 1.48 g/120 square cm / 28 days respectively). The ratio of secondary: primary follicles in all goats was 12.60.

**Table 1** Effects of feeding level on cashmere diameter during experiment period. (Means  $\pm$  standard deviation)

Feeding levels	April	May	June	July	August	September
0.7 M <sup>#</sup>	15.67 <sup>a</sup> $\pm$ 0.90	16.23 <sup>a</sup> $\pm$ 0.93	17.97 <sup>a</sup> $\pm$ 0.59	17.42 <sup>a</sup> $\pm$ 0.85	16.50 <sup>a</sup> $\pm$ 1.15	16.36 <sup>a</sup> $\pm$ 1.70
M	16.30 <sup>b</sup> $\pm$ 1.47	17.00 <sup>b</sup> $\pm$ 0.73	17.62 <sup>a</sup> $\pm$ 0.83	18.01 <sup>b</sup> $\pm$ 0.70	17.41 <sup>b</sup> $\pm$ 1.26	17.90 <sup>b</sup> $\pm$ 1.10
1.4 M	16.01 <sup>b</sup> $\pm$ 1.41	16.60 <sup>b</sup> $\pm$ 1.22	18.00 <sup>b</sup> $\pm$ 0.76	18.09 <sup>b</sup> $\pm$ 0.83	17.78 <sup>b</sup> $\pm$ 0.91	17.86 <sup>b</sup> $\pm$ 0.86
1.8M	17.20 <sup>b</sup> $\pm$ 0.77	17.52 <sup>b</sup> $\pm$ 0.52	18.05 <sup>b</sup> $\pm$ 0.74	18.33 <sup>b</sup> $\pm$ 0.81	18.36 <sup>b</sup> $\pm$ 0.93	18.00 <sup>b</sup> $\pm$ 1.35

<sup>#</sup> M=Maintenance.

Comparable means within a column with different superscripts differ significantly ( $P < 0.05$ )

**Conclusion** Feeding more than maintenance requirements resulted in no increase in cashmere production. Raising cashmere goats in large scale and under extensive production system must be studied in Tabriz climate in future studies.

**Acknowledgments** This experiment was contacted under financial support of Research and Technology Office of University of Tabriz.

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## The effect of forage maize hybrid type on *in vivo* and *in vitro* dry matter digestibility

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**Introduction** The digestibility values of forages have long been recognised as an important parameter affecting both milk yield and growth of ruminants. Several methods (*in vivo* and *in vitro*) are used to determine digestibility of forages. The aim of this experiment was to determine the effect of chemical composition of silages on the *in vivo* and *in vitro* DMD of maize silages.

**Materials and Methods** Four maize forages from different hybrids (Nancis, Ema, 6196 and Volgate) were ensiled in sealed drums with no additives. The *in vivo* DMD values of the resultant silages were determined using fistulated suffolk X wethers in a 4X4 factorial design. Sheep were fed twice daily with total intake of 900 g dry matter (DM) of silage plus 150g Soya Bean Meal. After an adaptation (21 days) a 5 day total collection of faeces was carried out. The *in vitro* DMD values were determined according to the two -stage rumen inoculum-pepsin method (Tilley and Terry, 1963). Statistical analysis was carried out by ANOVA using GLM on Minitab.

**Results** Analysis of the four silage sources (Table 1) showed that there was considerable variation between silages in terms of chemical composition. Nancis and Ema had a low starch content when compared with 6196 and Volgate whereas ADF content for Nancis and Ema was higher than that for 6196 and Volgate.

**Table 1** Chemical composition of four maize silages

Silages	DM	Starch (g/kg DM)	WSC (g/kg DM)	Protein (g/kg DM)	Oil ext. (g/kg DM)	NDF (g/kg DM)	ADF (g/kg DM)	Ash	pH
Nancis	270	245	79	86	28	410	266	61	3.5
Ema	250	250	80	96	29	383	266	43	3.6
6196	420	348	50	85	53	353	220	45	3.9
Volgate	580	308	37	86	30	384	215	37	4.4

The *in vivo* and *in vitro* DMDs are given in Table 2. As can be seen from Table 2 Nancis with a low starch and high WSC content had a higher digestibility value when compared with the other silages although it had a high NDF and ADF content. On the other hand 6196 and Volgate had a low digestibility value although they had a high starch and low WSC content which is quickly available to rumen microorganism. The *in vitro* DMD values higher than the *in vivo* DMD values for all silages. There was a significant correlation ( $r=0.827$ ,  $n=16$ ,  $P<0.05$ ) between *in vitro* and *in vivo* DMD.

**Table 2** *In vivo* and *in vitro* dry matter digestibility

DMD	Nancis	Ema	6196	Volgate	SEM	Sig
<i>In vivo</i>	0.754 <sup>a</sup>	0.714 <sup>b</sup>	0.714 <sup>b</sup>	0.714 <sup>b</sup>	0.004	***
<i>In vitro</i>	0.787 <sup>a</sup>	0.753 <sup>b</sup>	0.740 <sup>b</sup>	0.745 <sup>b</sup>	0.007	***

Means within the same row with differing superscripts are significantly different. \*\*\*-  $P<0.001$

**Conclusion** The differences in chemical composition between the silages had an effect on the *in vitro* and *in vivo* DMD. A significant correlation between *in vivo* and *in vitro* DMD values indicated that it is possible to use the two -stage rumen inoculum-pepsin method (Tilley and Terry, 1963) for predicting *in vivo* DMD of maize silages

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## Tannin bioassay using semi-automated and manual gas production techniques for Brazilian browses

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**Introduction:** Native herbaceous browses at Northeast of Brazil have a dry tolerance and have been used as animal feed. Some of those plants have anti nutritional compounds such as tannins that can interfere on their intake and digestibility. Polyethylene glycol (PEG) has been used in gas-based techniques for assessing anti nutritional factors in tanniniferous plants for ruminants. The objective of this work was to compare the tannin bioassay technique using the semi-automated (Reading Pressure Technique - RPT) and manual (syringe) gas production techniques to evaluate the tannin effect upon *in vitro* rumen fermentation, using PEG as an inhibitor of tannin effects.

**Material and methods:** Seven plant species commonly found in Brazil were used as substrates for both RPT and syringe techniques. They were: *Anadenanthera macrocarpa*, *Cajanus cajan*, *Leucaena leucocephala*, *Mimosa tenuiflora*, *Myracrodruon urundeuva*, *Sesbania sesban* and *Sida cordifolia*. Total phenols (TP), total tannins (TT) and condensed tannins (CT) were determined (Makkar, 2000). RPT was carried out according to Mauricio et al. (1999) and syringe according to Menke et al. (1979). Four sheep with rumen cannulas were used as inoculum donors. Inoculum was prepared with 50% liquid and 50% particulate ruminal matter (Bueno et al., 2000). About one gram of each sample was disposed in triplicate for RPT, with or without PEG (1:1). For syringe technique 350 mg of samples were used in triplicate with or without 750 mg of PEG. Gas production was recorded at 3, 6, 9, 12, 16 and 24 h. Cumulative gas production after 24 h were compared for both RPT and syringes. PEG effect was determined based on gas production increment between samples with and without PEG. Gas production increments were compared to TP, TT and CT by correlation analysis.

**Results:** Crude protein (CP), TP, TT and CT contents are described on Table 1. RPT and syringes techniques showed PEG effect through increment of gas production. Pearson's coefficient values were higher for RPT than syringes for all correlations (Table 2), suggesting lower variation of gas measurements in RPT. The increments of gas production varied to 51.9 – 294.4 % for RPT and 2.9 – 39.8 % for syringes. Different forms of tannins molecules among these plants could explain the negative values in Pearson's coefficient between gas increments and CT concentrations (in RPT and syringes).

**Table 1** Chemical composition of Brazilian browses

Substrate	crude protein <sup>1</sup>	total phenols <sup>1</sup>	total tannins <sup>2</sup>	condensed tannins <sup>1</sup>
<i>Anadenanthera macrocarpa</i>	162	13	12	9
<i>Cajanus cajan</i>	138	3	2	6
<i>Leucaena leucocephala</i>	187	3	2	10
<i>Mimosa tenuiflora</i>	160	13	11	69
<i>Myracrodruon urundeuva</i>	130	19	18	43
<i>Sesbania sesban</i>	187	1	1	2
<i>Sida cordifolia</i>	135	7	5	97

<sup>1</sup>g.kg<sup>-1</sup>; <sup>2</sup>tannic acid (mg%)

**Table 2** Pearson's coefficients (r) for significant (P< 0.05) correlations between gas production increment and feed tannins composition of Brazilian browses

	RPT	Syringes	Total phenols	Total tannins	C. Tannins
RPT	-	0.37	0.58	0.59	-0.32
Syringes	0.37	-	0.05	0.03	-0.04
Total phenols	0.58	0.05	-	0.99	0.38
Total tannins	0.59	0.03	0.99	-	0.34
C. Tannins	-0.32	-0.04	0.38	0.34	-

**Conclusions:** Tannin bioassay using the RTP technique better reflected the TP and TT concentration in plants and their effect upon *in vitro* rumen fermentation. Syringe was also able to determine these effects, although it was not so sensitive to reflect variations on tannins concentrations of these plants.

**Acknowledgements:** This experiment is part of projects supported by FAPESP.

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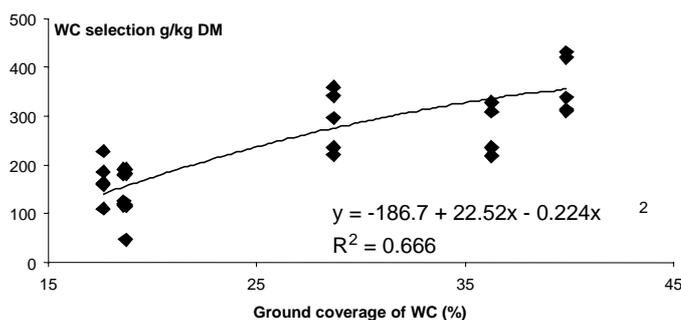
# Voluntary herbage intake and diet selection in Scottish-Blackface ewes suckling twin lambs and grazing perennial ryegrass/white clover swards with or without protein supplementation

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**Introduction** Mixed perennial ryegrass and white clover swards are often used as the basis of upland sheep grazing systems. This study's objective was to examine voluntary herbage intake and diet selection in 30 lactating Scottish-Blackface ewes grazing mixed perennial ryegrass (PRG) and white clover (WC) swards supplemented with or without protein. The work was part of a wider study of nutrition and parasitology in organic sheep systems.

**Materials and methods** A 2 x 3 factorial continuous randomised block design experiment was conducted to determine voluntary herbage dry matter intake (DMI) and diet selection in multiparous Scottish-Blackface ewes (mean LW 55.3 kg) suckling twin lambs. Experimental factors were level of protein supplementation (PROT) and herbage plot area (PLOT) which were designed specifically to vary in the proportion of PRG and WC available. All ewes were managed to achieve a body condition score (CS) of 2-2.25 at lambing. After lambing, ewes were paired and allocated to 2 dietary treatments according to lambing date, CS, liveweight (LW), flock of origin and faecal egg output. Dietary treatments were either grazing alone or grazing plus 0.6 kg/ewe/day of a non-GM soyabean meal (SBM). Thereafter, ewes were allocated at random to the 3 herbage areas, each of which was split into 2 sub-plots to allow for differential concentrate feeding. Herbage sward heights were maintained within a target range of 4-6 cm and average ewe stocking rates were 18 ewes/ha. Voluntary herbage DMI and diet selection for 30 ewes was determined using the *n*-alkane approach (Dove and Mayes, 1991) on one occasion during 20<sup>th</sup>-24<sup>th</sup> May 2002 when ewes were on average in week 5 of lactation. Exogenous *n*-alkanes (C32 and C36) were administered to each ewe using a Captec® bolus and herbage DMI calculated assuming daily dose rates published by Mayes *et al*, (1991). Diet selection for each ewe was determined from *n*-alkane concentrations in herbage and faeces samples using a non-negative least squares procedure (Newman *et al*, 1995). Ground coverage (%) of PRG and WC in the sward was determined using ten 0.5x0.5m quadrats per sub-plot. Intake and diet selection data were subjected to analysis of variance using Genstat 5 and the relationship between the proportion of dietary WC selected by ewes and the ground cover of WC was investigated using regression analysis.



**Figure 1.** Ground cover of WC and WC dietary selection.

**Results** PLOT influenced the DMI of WC ( $P < 0.05$ ) and feeding SBM increased ( $P < 0.05$ ) total DMI compared with grazing alone but there were no other treatment effects on DMI figures (Table 1) indicating that feeding SBM did not reduce herbage intake. Ewes grazing PLOT 2 also selected higher WC and lower PRG proportions ( $P < 0.001$ ) than the other two PLOTS. There was a positive quadratic relationship between the proportions of WC selected by individual ewes and the estimated % ground coverage of WC (Figure 1).

**Table 1.** Feed intakes and dietary proportions of PRG and WC in grazing organic Scottish-Blackface ewes.

		PROT			GRAZING PLOT			Sig of effects		
		None	SBM	sed	1	2	3	sed	PROT	PLOT
DMI	SBM	0	0.51		0.25	0.25	0.25			
(kg/d)	PRG	1.57	1.74	0.208	1.89	1.51	1.58	0.255		
	WC	<u>0.58</u>	<u>0.46</u>	0.068	<u>0.49<sup>a</sup></u>	<u>0.77<sup>b</sup></u>	<u>0.31<sup>c</sup></u>	0.084		*
	Total	<u>2.16</u>	<u>2.71</u>	0.242	<u>2.63</u>	<u>2.54</u>	<u>2.14</u>	0.297	*	
Total	g/kg LW	40.2	48.3	4.82	47.8	45.6	39.4	5.90		
Total	g/kg LW <sup>0.75</sup>	109	132	12.7	130	124	107	15.6		
Dietary proportion	PRG	740	787	24.7	790 <sup>a</sup>	658 <sup>b</sup>	843 <sup>a</sup>	30.2		***
(g/kg)	WC	260	213	24.7	210 <sup>a</sup>	342 <sup>b</sup>	157 <sup>a</sup>	30.2		***

For grazing PLOT, values not sharing common superscripts differ significantly ( $P < 0.05$ ).

**Conclusions** At sward heights of 4-6 cm, SBM supplement increased total DMI but did not influence dietary selection by grazing ewes or total herbage DMI. Dietary selection of WC by ewes was directly related to % cover in the sward.

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

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## Nutrient digestibility of alfalfa at different growth stages on sheep and goat

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**Introduction** The nutritive values of animal feed are dependents on plant species, stages of maturity, harvesting and preparation methods. Legumes provide maximum yield, high forage quality (protein, mineral and digestible energy). Legumes decrease in protein and digestible dry matter and increase in fibre as they increase in growth or in maturity (Hochensmith et al., 1997). Alfalfa (*medica sativa*) is world unique forage in livestock food. This study was conducted to examine the chemical composition and nutrient digestibility of Hamadani alfalfa forage at different growth stage on two local Iranian sheep and goat breeds.

**Materials and methods** Cultivars characteristics of alfalfa including, bush height, bush dry matter and their ratio were measured in four stages of growth (1- Budding time 2-Completed budding time 3-Early flowering time 4 - Completed flowering time). Chemical compositions of samples after harvesting from each stages of maturity were measured by methods described by Weende. Nutrient digestibility coefficients of alfalfa were obtained by using four Sanjabi rams (42±2.1) and four Merghoze buck (34±2.3) during spring in Kermanshah Province. Random experimental designs were used and data was analyzed using mean comparative T test and Duncan methods.

**Results** The mean of bush height, amount of dry matter and percentages of dry matter of fifty bushes in the budding time were 43 ± 3.9 cm, 29±4.8 g and 17.66±2.3 % respectively, whereas these parameters were 55.6±5.6 cm, 45. 8 ± 9.5 g and 23.1 ± 2.6 % for completed flowering time.

These results have shown that Hamadani alfalfa significantly includes more crude protein at budding time compared with completed flowering time (21±1.6 vs. 16±1.9 %), which is in contrast with fiber percentages (21±1.1 vs. 24±0.9%). Digestibility of dry matter (DM), crude protein (CP) and crude fiber (CF) at budding time were 63.5±2.1, 74.7±2.8 and 46.1±4.8 percent in sheep whereas these coefficients were 66.7 ±2.6, 76.4±3.1 and 48.1±4.1 percent in goat respectively (Table 1). There is a significant difference in crude protein at budding time compared with completed flowering time (p<0.05), but no significant effect has been found in DM and CF digestibility in the stages of growth. The errors associated with predicted digestibility depends on harvesting methods, the set and number of samples and technique used at laboratory analysis. There were no significant differences of digestibility between sheep and goat except on completed flowering time, which dry matter and crud protein digestibility were higher in goat (P<0.05).

**Table 1** Digestibility coefficients of alfalfa at different stages of growth (%)

	Growth stages	Budding Time	Completed Budding	Early Flowering	Completed Flowering	S.E.M.
Sheep	DM	63.5	62.4	59.1	58.8	2.01
	CP	74.7	72.1	68.8	67.8	3.53
	CF	46.1	42.7	36.5	37.5	3.88
Goat	DM	66.7	64.5	62.8	62.1	2.20
	CP	76.4	75.2	73.0	71.7	3.45
	CF	48.1	46.0	42.0	40.1	3.93

**Conclusions** It can be concluded that despite lower crop yield compare to early and completed flowering time, the high nutrient digestibility of alfalfa is at budding stage. The highest forage quality of Hamadani alfalfa has been obtained by harvesting forage at budding time.

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# Study on phosphorus metabolism in growing sheep fed different sources of calcium by using the isotope dilution technique

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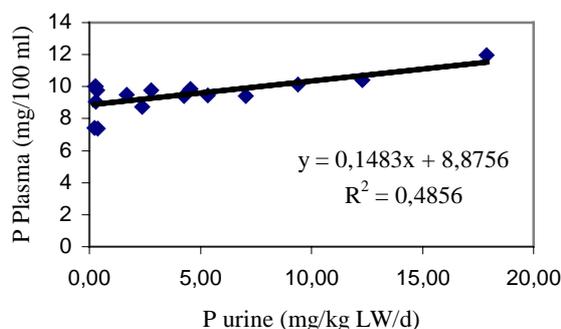
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**Introduction:** The close association of calcium and phosphorus in bone, and the narrow relationship between these minerals makes this subject always an important aim of study for researches on animal nutrition (Braithwaite, 1984). The utilization of alternative sources of calcium has been studied in Brazil in the last years however there is a lack of information about the effects of these sources on phosphorus metabolism. The aim of this study was to evaluate phosphorus metabolism in sheep fed four different sources of calcium through determination of true absorption and endogenous faecal loss of phosphorus by using the isotope dilution technique (Vitti et al., 2000).

**Materials and methods:** The study was carried out with sixteen brazilian breed male sheep, housed indoors in metabolism cages, receiving a basic diet supplemented with different sources of calcium during twenty-one days. These sources were: limestone, lucerne hay, citrus pulp and shell meal (T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> respectively). After 21d pre-experimental period a dose of 7.4 MBq <sup>32</sup>P was injected into the left jugular vein of each animal. Blood samples, feces and urine were collected every 24 hours for 7 days and analyzed for inorganic phosphorus and radioactivity. Experimental measurements were analyzed as a completely randomized design. General Linear Models Procedure (SAS, 1991) was used for comparison of means from each category with sources of variation being the animals in each treatment with a different source of calcium.

**Results:** The endogenous faecal loss was 50.61, 45.55, 44.26, and 36.87mg/kg LW/d for treatments 1, 2, 3 and 4 respectively (P>0.05). Phosphorus absorption was: 43.47, 48.89, 40.16 and 52mg/kg LW/d for T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> e T<sub>4</sub> respectively (P>0.05). The average P intake was 99.32, 128.46, 124.35, 137.88 mg/kg LW/d for T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> e T<sub>4</sub> respectively (P<0.05). Plasma P concentration had high values for all treatments: 10.23, 9.83, 8.41 and 9.45mg/100ml respectively for T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> e T<sub>4</sub> (P>0.05) and P in urine was 9.65, 0.645, 0.925 and 6.14 mg/kg LW/d for T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> respectively (P<0.01). Urinary loss of P had a linear relationship with P in plasma:  $y = 0.1483x + 8.8756$  (n=16, r<sup>2</sup> = 0.4856, P<0.01) as shows in figure 1. The high values for P plasma may reflect an excess of P intake, being excreted in urine mainly for T<sub>1</sub> and T<sub>4</sub>. Phosphorus was absorbed from the T<sub>2</sub> and T<sub>3</sub> diet at 36% and 31% efficiency respectively, and absorption for T<sub>1</sub> and T<sub>4</sub> diets was at 41% and 39% efficiency, respectively. The lower efficiency of phosphorus absorption observed for T<sub>2</sub> and T<sub>3</sub> may reflect the low availability of phosphorus, mainly present in the organic form.

**Fig. 1:** Relationship between P in urine and P in plasma for sheep given different treatments



**Table 1:** Means of variables: endogenous faecal loss (E.F.L.; mg/kg LW/d), absorbed P (A.P.; mg/kg LW/d), P intake (P.I.; mg/kg LW/d), plasma P (P.P.; mg/100ml) and urinary P (U.P.; mg/kg LW/d).

	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	S.E.M.
E.F.L.	50.61	45.55	44.26	36.87	7.529
A.P.	43.47	48.89	40.16	52	14.495
P.I.	99.32 <sup>a*</sup>	128.48 <sup>ab*</sup>	124.35 <sup>ab*</sup>	137.88 <sup>b*</sup>	9.912
P.P.	10.23	9.83	8.41	9.45	0.625
U.P.	9.65 <sup>a**</sup>	0.645 <sup>b**</sup>	0.925 <sup>b**</sup>	6.14 <sup>ab**</sup>	1.857

<sup>a, b</sup> Mean values with unlike superscript letters were significantly different  
(\*) P < 0.05 and (\*\*) P < 0.01.

**Conclusions:** The results show that there was not a significant difference in phosphorus absorption and the endogenous faecal loss between treatments however efficiency of phosphorus absorption was higher for T<sub>1</sub> and T<sub>4</sub>. Urinary excretion of phosphorus may have had a role on phosphorus homeostasis by eliminating the surplus P.

**Acknowledgements:** This experiment is part of a project supported by CNPq.

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# The effect of dietary molybdenum or iron on copper status and trace element accumulation in the pituitary and ovary of growing lambs

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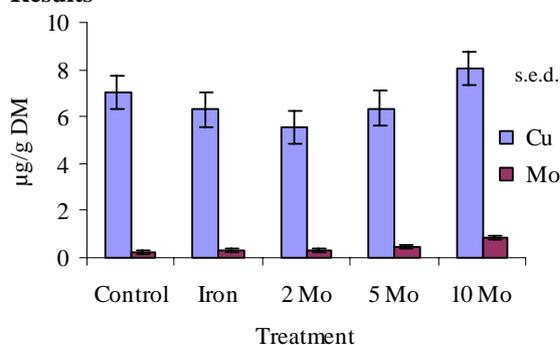
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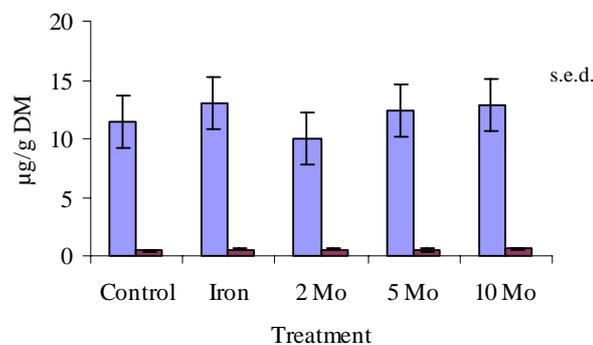
**Introduction** Secondary copper deficiency in ruminant animals is induced by high dietary levels of molybdenum (Mo), iron (Fe) or sulphur (S). Within the rumen, sulphur reacts with Mo to form a series of thiomolybdate molecules (TM) which may chelate copper. This reduces copper absorption or if TM is absorbed, inhibits copper metallo-enzyme activities. Parental administration of TM has resulted in an increase in Cu to the brain and an increase in Mo to the pituitaries (Haywood *et al.*, 1998). This redistribution may alter neurological, endocrine and reproductive function. However, there are no reports on effects of endogenously produced TM on brain or pituitary trace element accumulation. The objective of this study was to determine the effect of dietary Mo or Fe on copper status and mineral retention in the pituitary gland and ovary of growing lambs.

**Materials and Methods** Fifty Charollais cross female lambs with an initial mean liveweight of 22.9kg (s.e.d. 1.4) were individually penned and randomly allocated to one of five dietary treatment groups. All lambs were fed a restricted basal complete diet (CP 134 g/kg DM; ME 10.7 MJ/kg DM; 6.09 mg/kg Cu; 0.94 mg/kg Mo). Treatment one (Control) received a basal diet. Treatment two (Fe) received the basal plus an additional 500 mg/kg DM Fe and 2 g/kg S and treatments 3 (2 Mo), 4 (5 Mo) and 5 (10 Mo) received the basal diet in addition to 2, 5 or 10 mg/kg DM Mo and 2g/kg S respectively. Blood samples were obtained fortnightly for ceruloplasmin (CP), plasma copper (PI-Cu) and superoxide dismutase (SOD) activity for 12 weeks. Pituitary glands and ovaries were collected at slaughter (week 12) and mineral content was determined by ICP-MS. Statistical analysis was performed using ANOVA using Genstat version 6.

## Results



**Figure 1** Effect of treatment on mineral accumulation in the ovary



**Figure 2** Effect of treatment on mineral accumulation in the pituitary gland

Ovary Cu content was significantly lower ( $P < 0.05$ ) in the 2 Mo treatment group compared to the control or 10 Mo treatments (Fig. 1). Ovary Mo content was significantly increased ( $P < 0.01$ ) by Mo supplementation (Fig 1). There was no significant effect of treatment on copper or molybdenum accumulation in the pituitary gland (Fig 2.).

**Table 1** Effect of treatment on copper status

	Wk	Con	Fe	2 Mo	5 Mo	10 Mo	s.e.d.	Sig
PI-Cu µmol/L	0	14.57	15.38	14.48	14.83	14.32	1.200	NS
	6	10.78	10.16	10.39	10.78	13.22	0.957	*
	12	11.72	10.68	11.27	11.35	16.69	0.843	***
CP mg/dL	0	20.70	23.12	19.52	19.03	19.50	2.178	NS
	6	12.49	13.99	12.31	10.73	13.01	1.207	NS
	12	15.11	14.68	13.03	10.59	13.89	1.449	*
SOD U/g Hb	0	2040	2223	1945	2139	2038	173	NS
	6	1058	1105	1030	964	1111	107	NS
	12	1266	1182	1256	1126	1080	110	NS

PI-Cu levels were significantly higher ( $P < 0.05$ ) in the 10 Mo group at weeks 6 and 12 (Table 1).

There was a trend for CP activity to decrease throughout the trial period with the 5 Mo treatment having significantly lower CP activity by week 12 ( $P < 0.05$ ) (Table 1).

There was no significant effect of treatment on SOD activity by wk 12 (Table 1).

**Conclusion** Increased dietary Mo resulted in the retention of Mo in the ovary but not the pituitary gland. TM formed within the rumen may be the mechanism by which the redistribution of Cu or Mo to various target organs occurs. Accumulation of Mo within the ovary may have implications regarding the reduction in fertility seen in animals given high dietary Mo. Dietary Mo did not have pronounced systemic effects on copper dependent enzyme activities.

**Acknowledgement** The Silcock Foundation for the funding of this work.

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## A phosphorus metabolism model for sheep fed various levels of calcium

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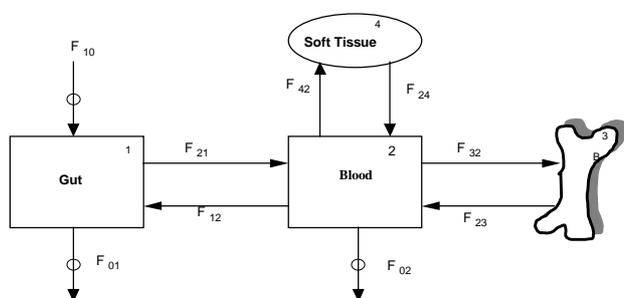
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**Introduction** In tropical countries, especially in Brazil, ruminants commonly are fed forage diets which are adequate in calcium but deficient in phosphorus, giving an inadequate Ca: P ratio. Thus, it is important to understand the effects of Ca level on phosphorus homeostasis control and to obtain more information on phosphorus utilization. The objective of this study is to provide information which may be useful to improve the understanding of the P homeostasis control in sheep fed various Ca levels, and to obtain more information on dietary Ca level effect on P utilization.

**Material and methods** During a 28 days period, nine castrated Suffolk sheep received rations, based on hay, (8.7%CP;2199 Mj/kg ME) containing different proportions of Ca and P: Treatment 1=0.75:1, Treatment 2=1.5:1 and Treatment 3=3:1 (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 phosphorus and radioactivity in all the samples were measured. After the end of collection period tissues samples were collected (liver, heart, kidney, muscles and 12<sup>th</sup> rib) for analysis. Experimentally measured (model inputs) and model output were statistically analyzed. The data used in the analysis were from 9 animals, 3 from treatment. 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 sheep in each treatment and Ca:P rate. Treatment means were assessed for significant differences at  $P < 0.05$ .

**Results** Figure 1 shows the schematic representation of the model of phosphorus metabolism. The daily P supply was kept constant and represented an adequate level (84mg/kg live weight per day). The daily Ca supply was deficient (60.86 mg/kg live weight per day), adequate (124 mg/kg live weight per day) and excessive (241 mg/kg live weight per day), respectively for treatments 1, 2 and 3. (Challa & Braithwaite, 1989). Phosphorus excretion in faeces ( $F_{01}$ ) and urine ( $F_{02}$ ) (Table 1) were similar for all treatments ( $P > 0.05$ ). Phosphorus in blood, bone and soft tissues did not showed differences between treatments. Total phosphorus absorption ( $F_{21}$ ) and phosphorus flow from the central pool to gut ( $F_{12}$ ) were not influenced by Ca levels ( $P > 0.05$ ). However, there was a tendency of increasing values for treatment 2 (1.5:1). Phosphorus recycling from blood to bone ( $F_{32}$ ) showed not differences between treatments as well the flow of phosphorus from blood to tissues ( $P > 0.05$ ). However, the values for treatment 2 (11.11) show a tendency of increasing in relation to the other treatments. Calcium intake did not affect the phosphorus outflow from bone and tissues to the central pool.



**Table 1** - Comparison of kinetic model input and outputs (g/day) for the different Ca: P ratios in sheep

		Treatments			S.E.M.
		1	2	3	
<b>Input fluxes</b>					
Intake	F10	3.00	3.00	3.00	0.000
Faeces	F01	2.64	2.65	2.72	0.076
Urine	F02	0.01	0.01	0.01	0.003
<b>Model output</b>					
Blood P to gut	F12	3.37	4.76	3.92	0.717
P absorption	F21	3.74	5.11	4.20	0.709
Blood P to bone	F32	1.09	1.06	0.91	0.237
Blood P to tissue	F42	7.58	11.1	9.26	1.371
Tissue P to bone	F24	1.62	2.21	1.78	0.209
Bone P to blood	F23	6.70	9.61	8.12	1.396

**Conclusions** From the results it can be suggested that calcium intake level may affect the flow of phosphorus from the central pool to tissues as well the phosphorus absorption from gut and the phosphorus output from the central pool to gut. However, the lack of a clear response in phosphorus metabolism at the calcium levels used in the present study, suggest that wider Ca: P ratios should be used to verify such effects. The kinetics model could be used to illustrate the different processes that occur in sheep fed various Ca and P rates.

**Acknowledgements** This experiment is part of a project supported by FAPESP.

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# The effects of mineral block ingredients when offered to ewes in late pregnancy on Immunoglobulin G (IgG) absorption in their lambs

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**Introduction** Lambs are born hypoinmunocompetent as the placenta prevents the transfer of maternal immunity to the foetus of the ruminant. Colostrum is the source of immunoglobulins for the young lamb and any interference with the absorption of immunoglobulins from colostrum would have important consequences for lamb liveability in early life. Recent experiments at this institute found that when ewes had access to mineral blocks in late pregnancy the absorption of immunoglobulin (IgG) by their offspring was reduced (Keane 2001). This author also stated that the lamb was pre-programmed in-utero for lowered IgG absorption efficiency. The aim of this experiment was to determine whether it was the mineral or molasses component of the mineral block which caused the reduced IgG absorption by the lamb.

**Materials and Methods** Following scanning, 90 twin-bearing ewes were selected at day 99 of gestation and offered a basal diet of grass silage ad libitum plus 435g of a 19% crude protein concentrate. Five experimental treatments were imposed consisting of the basal diet plus one of the following: no supplement (C), mineral block (B) (100g/d), molasses (MS), minerals (ML) or minerals + molasses (ML+MS). The MS and ML treatments got the same amount of molasses and minerals respectively as in 100g of block. Silage and water intakes were recorded daily. Silage was fed twice daily at 0900 hrs and 1700 hrs and concentrates were fed at 1000 hrs each day. All ewes were individually fed. Ewes lambing between days 143-152 of pregnancy were hand milked at 1, 10 and 18 hours post partum following administration of 10 i.u. of Oxytocin. Colostrum samples were taken for IgG determination. Lambs were blood sampled by venipuncture using a 5ml vacutainer 24 hours post partum. The IgG content of colostrum was measured by the method of Fahey and McKelvey (1965) using single radial immunodiffusion (RID) kits (Bethyl Laboratories, Montgomery, Texas). Lamb serum was assayed for total immunoglobulin level using the zinc sulphate turbidity test (McEwan et al., 1970).

**Results** Ewe and lamb performance data are given in Table 1. Treatment had no significant effect on total dry matter intake ( $P>0.05$ ). Ewes in treatment B had a higher ME intake than controls (C). Animals receiving both minerals and molasses, either as a block (B) or in the loose form (ML+MS) had higher water intakes than in any other treatment ( $P<0.01$ ). Treatment had no effect on colostrum yield to 18 hours or total IgG yield to 18 hours ( $P>0.05$ ). In the treatments where ewes were offered minerals, either in the block (B) or added to the concentrate (ML, ML+MS) their progeny had a significantly ( $P<0.001$ ) lower percentage of colostrum IgG absorbed and lower blood serum IgG concentration at 24 h post lambing. Lamb weaning weight and growth rate to weaning was lower in the ML+MS treatment than in treatments C, B and MS ( $P<0.05$ ).

**Table 1** The effects of mineral block ingredients on ewe and lamb performance (L.S.M.  $\pm$  S.E.M.)

Treatment	C	B	MS	ML	ML+MS	SEM
Total dry matter intake (kg)	1.23	1.34	1.29	1.28	1.26	0.042
ME intake (MJ)	13.17 <sup>a</sup>	15.12 <sup>b</sup>	14.78 <sup>ab</sup>	13.57 <sup>ab</sup>	14.35 <sup>ab</sup>	0.630
Water intake (l)	4.34 <sup>a</sup>	6.38 <sup>c</sup>	4.63 <sup>a</sup>	4.83 <sup>a</sup>	5.59 <sup>b</sup>	0.272
Colostrum yield to 18 hours (ml)	1645	1830	1670	1631	1634	142.35
Total IgG yield to 18 hours (g)	84.78	85.37	74.32	77.35	72.28	7.18
Total IgG fed to 18 hours (g)	30.80 <sup>b</sup>	30.41 <sup>b</sup>	26.01 <sup>a</sup>	29.69 <sup>ab</sup>	26.89 <sup>ab</sup>	1.62
Lamb serum IgG at 24h (g/l)	25.02 <sup>d</sup>	11.30 <sup>b</sup>	21.18 <sup>c</sup>	2.91 <sup>a</sup>	3.36 <sup>a</sup>	1.41
% Colostral IgG absorbed	26.49 <sup>c</sup>	12.20 <sup>b</sup>	28.76 <sup>c</sup>	3.33 <sup>a</sup>	5.07 <sup>a</sup>	1.54
Weaning weight (kg)	32.19 <sup>b</sup>	32.24 <sup>b</sup>	32.79 <sup>b</sup>	30.47 <sup>ab</sup>	29.33 <sup>a</sup>	0.983
Growth rate, birth to weaning (g/day)	264 <sup>b</sup>	264 <sup>b</sup>	269 <sup>b</sup>	247 <sup>ab</sup>	236 <sup>a</sup>	9.30

Means within rows with same superscripts are not significantly different

**Conclusions** The dietary supplementation of individually fed ewes in late pregnancy with minerals plus molasses resulted in increased water intake. Supplementing the diet of ewes with the mineral component of mineral blocks at the level used in the current experiment resulted in reduced blood serum IgG values in the lamb and a lowered efficiency of IgG absorption at 24 h post partum. Further studies are required to determine the specific ingredient(s) in mineral blocks responsible for this lowered efficiency of IgG absorption.

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## The intake and digestibility in Raini male goats fed different ratios of effective rumen degradable nitrogen:sulphur

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**Introduction** Nutrient deficiencies, such as sulfur (S), that reduce the activities of rumen microorganisms are liable to reduce food intake (McDonald *et al.*, 1995). Hegarty *et al.* (1994) found that a depression of appetite and digestibility in sheep deprived of sulfur. The amount of sulfur needed to optimize the rumen environment is dependent on several factors such as the level of dietary sulfur and the availability of the effective ruminal degradable nitrogen, ERDN (Underwood and Suttle, 1999). Therefore, this trial was conducted to assess the influence of different ERDN:S ratios on the dry matter intake (DMI) and nutrients digestibility in the male goat of Raini breed, an Iranian native cashmere goat.

**Material and Methods** Daily, the DMI was measured using forty goats with an initial live weight of 19.0 Kg (sd 2.15) and aged 9 months. The animals which were individually penned were randomly assigned into five groups (n=8) for a period of 98 days. A completely randomized design was used with five rations containing either 0.14, 0.22, 0.28, 0.34 or 0.4 % of sulphur (dry matter basis). Calcium sulfate served as the source of supplemental S and was included to achieve ERDN:S ratios of 12:1, 7.6:1, 5.9:1, 4.9:1 and 4.1:1. The basal diet, which was based on barley, cottonseed hulls, and Lucerne hay, was formulated according to AFRC (1998). The metabolisable energy and metabolisable protein for the basal diet were 9.57 MJ/Kg DM and 84.2 g/Kg DM respectively. The animals were fed twice daily to appetite. At the end of the trial, fifteen animals were divided into five groups (n=3). These animals were transferred to metabolism crates. Total collection of faeces was conducted to quantify the digestibilities of dry matter (DMD), organic matter (OMD and the gross energy (GED). The data were statistically analyzed using a completely randomized design.

**Results** Table 1 shows the results. The ERDN: S ratio tended to improve the dry matter intake quadratically of the goats fed the experimental diets. However, this ratio had quadratically increases (P<0.05) the DMD, OMD and GED. Using the regression analysis between the ratio of ERDN: S and the DMI and nutrients digestibility the optimum ratio was 6.4:1.

**Table 1** The effect of different ERDN:S ratios on the DMI and nutrients digestibilities of goats

	Diets					S.E.	Sig.
	12:1	7.6:1	5.9:1	4.9:1	4.1:1		
Dry matter intake (g/day)	920	964	940	924	924	14.0	ns
Dry matter intake (g/kg W <sup>0.75</sup> )	76.2	76.4	76.9	75.9	78.1	1.6	ns
Dry matter digestibility (%)	50.8 <sup>c</sup>	54.4 <sup>a</sup>	53.1 <sup>b</sup>	51.2 <sup>c</sup>	51.0 <sup>c</sup>	0.13	**
Organic matter digestibility (%)	51.2 <sup>d</sup>	55.0 <sup>a</sup>	53.2 <sup>b</sup>	52.0 <sup>c</sup>	51.4 <sup>d</sup>	0.12	**
Gross Energy digestibility (%)	50.5 <sup>c</sup>	54.8 <sup>a</sup>	53.8 <sup>b</sup>	51.6 <sup>c</sup>	51.0 <sup>d</sup>	0.12	**

<sup>a, b, c</sup> Means in a same row with different superscripts differ significantly (P<0.05); \*\*significant at (P<0.01).

**Conclusion** The maximum intake and nutrients digestibility for the Raini male kids may be obtained at the dietary ERDN: S ratio of 6.4:1.

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## The influence of different levels of fish meal on the performance and carcass characteristics of Raini goat

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**Introduction** Earlier investigations have been carried out with Raini goat for maximizing the ruminal microbial protein production by determining the requirement of the dietary ERDN:S ratio. Adding a supplement which contains high level of undegradable protein (UDP) may improve the animal performance, due to the increase the of the amino acids concentration in the small intestine (Orskov, 1992). Therefore, this trial was conducted to maximize the potentiality of the growth performance and carcass characteristics of this breed by adding a protein supplement, fish meal (FM), which contains a high percent of undegradable protein.

**Material and Methods** Twelve Raini male goats with an initial live weight of 30 Kg (sd 2.5) and aged 2 years were used in this experiment. The animals, which were individually penned, were divided into four groups (n=3). The basal diet, which was based on barley, wheat straw, and Lucerne hay, was formulated according to AFRC (1998). The metabolisable energy and metabolisable protein for the basal diet were 10.04 MJ/Kg DM and 27.5g/Kg DM respectively. To each group FM was added at either 0, 50, 75 or 100 g FM per day. The animals which were fed *ad libitum* were weighed fortnightly for measuring the live weight gain (LWG). The duration of the trial lasted for 84 days. The dry matter intake (DMI) of the feed was measured daily. At the end of the trial, all animals were slaughtered and their carcasses were characterized. The data were statistically analyzed using a completely randomized design. Due to the difference in the initial weight of the animals, the initial weight was used as a covariate for all the analysis.

**Results** Table 1 shows the results. The FM supplement had no effect on the DMI, hot carcass, cold carcass and the viscera and intestines of the goats. However, it had significantly ( $P<0.05$ ) improved the LWG and feed conversion efficiency of the animals. The live weight gain which has been obtained in the animals fed the dietary supplement has been spread between the carcass and viscera and intestines weight.

**Table 1** The effect of FM on the performance and carcass characteristics of Raini goat

	level of fish meal (g/day)				S.E.
	0	50	75	100	
Initial Live weight (kg)	30.2	27.5	32.2	29.3	
Live weight gain (g/day)	43.7 <sup>a</sup>	67.5 <sup>b</sup>	79.8 <sup>b</sup>	97.2 <sup>c</sup>	0.53
Dry matter Intake (kg/day)	1.13	1.13	1.4	1.1	0.14
Feed conversion efficiency (DMI/LWG)	27.9 <sup>a</sup>	17.0 <sup>b</sup>	14.4 <sup>b</sup>	11.6 <sup>b</sup>	2.52
Hot carcass weight (kg)	16.2	15.6	18.3	16.6	0.31
Cold carcass weight (kg)	15.7	14.8	17.3	16.1	0.31
The viscera and intestines weight (kg)	9.15	10.2	11.3	11.4	0.33

<sup>a, b, c</sup> Means in a same row with different superscripts differ significantly ( $P<0.05$ )

**Conclusion** Adding FM to the diet of the Raini goat breed at this age or weight has improved the LWG as well as FCE.

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# Determination of colour on pork muscles by near infrared reflectance spectroscopy

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**Introduction** Colour is an important component of meat quality. Because colour is associated with factors such as freshness and food safety, it is often a primary consideration of consumers when making purchasing decisions (McCaig, 2002). The aim of this work was to explore the use of near infrared reflectance spectroscopy to assess colour composition on pork muscle samples from different feeding regimes.

**Materials and Methods** Samples from the loin (*longissimus thoracis*) (10 th rib) of 44 (90 - 110 kg live weight) carcasses were obtained 24 hours after slaughter. The samples were from a feeding experiment where T1: 100% commercial feed (n= 11), T2 and T3 combinations of different levels of commercial feed and pastures (n= 22) and T4 100% pastures (n= 11) for finishing pigs. The samples were thawed at room temperature (24 °C) during 10 hours and homogenised using a food processor. Colour readings were made using a Minolta camera (Minolta, Osaka, Japan) in triplicate. CIELab measurements performed were L (lightness of the colour), a (red and green characteristics) and b (yellow and blue characteristics) (McCaig 2002). Intact samples (approx. 100 g) were scanned in the visible and near infrared region on reflectance mode (400 – 2500 nm) in a scanning monochromator NIRS 6500 (NIRSystems, Silver Spring, MD, USA) in a large natural product cell. Homogenised samples were scanned using a quarter cup cell with disposable paperback. All samples were scanned once. Reflectance data were stored as log (1/R) at 2 nm intervals (where R is reflectance). Predictive equations were developed using modified partial least squares (MPLS) (Shenk and Westerhaus, 1993) regression with internal cross-validation (NIRS 2, 1995) and scatter correction using standard normal variate transformation (SNV) and detrending (Barnes et al., 1989). Three mathematical treatments were explored (1,4,4,1), (2,5,5,2) and (2,10,10,2). The optimum calibrations were selected based on minimising the standard error of cross validation (s.e.c.v.).

**Results** Table 1 shows the NIRS calibration and cross validation statistics for CIE L, CIE a and CIE b on both intact and homogenised muscles, respectively. The best  $r^2$  and s.e.c.v were 0.40 (4.8), 0.93 (1.30) and 0.60 (1.2) for CIE L, CIE a and CIE b on intact samples. The best  $r^2$  and s.e.c.v were 0.72 (3.6), 0.95 (1.4) and 0.24 (1.6) for CIE L, CIE a and CIE b on the homogenised samples.

**Table 1.** NIRS calibration statistics for CIE L, CIE a and CIE b on both intact and homogenised pork muscle samples (as percent).

	n	Mean	SD	$R^2_{cal}$	SEC	Math Treatment
<b>INTACT</b>						
CIE L	43	48.7	5.5	0.40	4.3	1,4,4,1, SNVD
CIE a	40	6.9	2.2	0.93	1.2	2,5,5,2, SNVD
CIE b	40	8.6	1.5	0.60	1.1	1,4,4,1, SNVD
<b>HOMOGENISED</b>						
CIE L	42	48.4	5.6	0.72	3.5	1,4,4,1, SNVD
CIE a	39	6.8	2.1	0.95	1.3	2,5,5,2, SNVD
CIE b	42	8.7	1.6	0.24	1.6	2,5,5,2, SNVD

**Conclusions** NIRS shows the capability to be used as a tool to estimate colour parameters on pork samples. The CIE a value was the best parameter predicted in both presentations. More samples will be included to improve the accuracy of the calibrations for the CIE L and CIE b parameters. Determination of colour on pork samples as well as chemical characteristics will improve the potential of NIRS as analytical tool in the meat industry.

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# Influence of grass silage fermentation and concentrate composition on the appearance and sensory characteristics of bovine *M. longissimus dorsi*

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**Introduction** Satisfying consumer preferences is an important element in ensuring the sustainability of the beef industry. An array of feed ingredients are available to beef producers but the effects of pre-slaughter ration composition on determinants of consumers' decisions to purchase beef, such as appearance and sensory characteristics, are unclear. Previous research has shown that the diet of cattle can influence fat colour, but that concentrates *per se* had little effect on sensory attributes of beef when compared with a grass silage or grass-based ration (French *et al.*, 2000). The objective of the current study was to examine the effect of silage fermentation and concentrate composition on the appearance and eating quality characteristics of beef.

**Materials and Methods** Groups of fifteen Friesian steers were individually offered either: extensively fermented unwilted grass silage (25 kg molasses/t grass) (EF), restricted fermentation, unwilted grass silage (91 (48% formic acid, 16% ammonium tetraformate/l)/t grass) (RF), a starchy concentrate (895 g barley/kg) (SC), a fibrous concentrate (880 g unmolassed sugarbeet pulp/kg) (FC), or zero-grazed perennial ryegrass (G). Concentrate allowances were restricted and offered with 1 kg wheat straw, per animal daily. The forages were supplemented with a minimum quantity of a common concentrate to ensure similar carcass growth for all groups. After 140 days, animals were slaughtered. Samples of subcutaneous fat and the *longissimus dorsi* (LD) muscle were removed 24 h post-mortem. Fat colour was then assessed. The pH of LD was measured after 2 days ageing post-mortem. Colour, Warner Bratzler shear force (an instrumental measurement of toughness) and sensory perception (trained taste panel) of LD were measured after 2, 7 and 14 days ageing post-mortem. Data were subjected to analysis of variance appropriate for a randomised block design.

**Results** The daily intakes of forage and concentrate dry matter were 7.9 kg and 3.46 kg, 6.98 kg and 3.46 kg, 0.84 kg and 6.98 kg, 0.84 kg and 7.60 kg and 9.4 kg and 1.79 kg for EF, RF, SC, FC and G, respectively. The corresponding carcass weights were 313, 318, 324, 313 and 324 (sed 7.7, ns) kg, respectively. Meat quality data are summarised in Table 1. Fat from animals offered G was more yellow (higher Hunter "b" value) than fat from animals offered RF, SC or FC. Neither LD pH, nor colour or shear force after 2, 7 or 14 days ageing differed between rations. For 2-day aged beef, the trend was for panellists to rate RF as most tender, juicy, flavoursome and acceptable, but the relative effect of ration composition was dependent on the attribute examined. After ageing for 14 days, the only difference between rations was that beef from EF was rated more juicy than that from RF, SC or G but not different from FC.

**Table 1** Fat colour and sensory characteristics of *M. longissimus dorsi*

	Silage		Concentrate		G <sup>1</sup>	sed	Sig
	EF <sup>1</sup>	RF <sup>1</sup>	SC <sup>1</sup>	FC <sup>1</sup>			
Fat Colour (Hunter "b")	20.2 <sup>a,b</sup>	19.5 <sup>b</sup>	19.0 <sup>b</sup>	18.8 <sup>b</sup>	21.3 <sup>a</sup>	0.73	**
Muscle <sup>2</sup>							
Tenderness - 2 days	3.89 <sup>a,b</sup>	4.64 <sup>a</sup>	4.20 <sup>a</sup>	4.13 <sup>a,b</sup>	3.35 <sup>b</sup>	0.415	*
Juiciness - 2 days	5.01 <sup>b</sup>	5.53 <sup>a</sup>	5.09 <sup>a,b</sup>	4.72 <sup>b</sup>	5.01 <sup>b</sup>	0.240	*
Flavour - 2 days	3.75	3.94	3.71	3.81	3.64	0.110	P=0.09
Acceptability - 2 days	2.80 <sup>a,b</sup>	3.25 <sup>a</sup>	3.03 <sup>a</sup>	3.10 <sup>a</sup>	2.56 <sup>b</sup>	0.234	*
Tenderness - 14 days	6.28	5.84	5.91	5.97	5.41	0.331	ns
Juiciness - 14 days	5.41 <sup>a</sup>	4.90 <sup>b,c</sup>	4.53 <sup>c</sup>	5.07 <sup>a,b</sup>	4.75 <sup>b,c</sup>	0.266	*
Flavour - 14 days	4.06	4.04	4.01	3.97	4.02	0.108	ns
Acceptability -14 days	3.86	3.75	3.78	3.57	3.64	0.167	ns

<sup>1</sup>EF = Extensive fermentation, RF = Restricted fermentation, SC = Starch, FC = Fibre, G = Grass; <sup>2</sup>For tenderness, 1 = extremely tender, 8 extremely tough; for juiciness, 1 = extremely dry, 8 = extremely juicy; for flavour, 1 = very poor, 6 = extremely good; for acceptability, 1 = not acceptable, 6 = extremely acceptable.

**Conclusions** These data provide guidance to beef producers formulating rations for cattle destined for markets with a fat colour specification. The lack of evidence for a general effect of pre-slaughter ration composition on sensory attributes of 14-day aged beef indicates that producers can choose the most cost-effective ingredients without concern for subsequent deleterious effects on beef appearance and eating quality.

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## Dietary manipulation of the polyunsaturated to saturated and *n-6:n-3* fatty acid ratios in lamb

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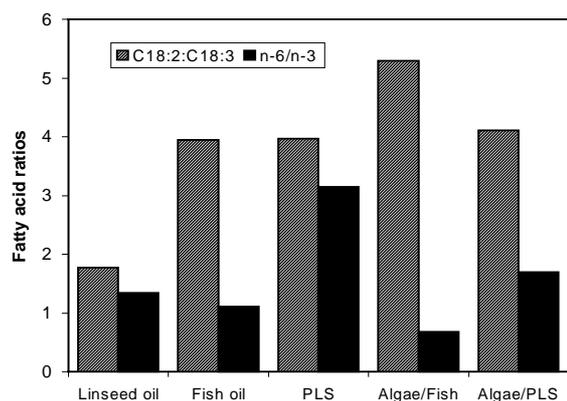
**Introduction** Lamb is characterized as having a low ratio of polyunsaturated (PUFA) to saturated fatty acids (P:S ratio) of approximately 0.1-0.2; considerably less than the minimum recommended ratio of 0.45 for the human diet as a whole (Department of Health 1994). Offset against this, lamb has a favourable ratio of the beneficial PUFA of the *n-3* series with the *n-6/n-3* ratio being well within the recommended value of <4.0, and is a particularly rich source of the longer chain *n-3* PUFA. One of the major obstacles to improving the P:S ratio of lamb is the extensive biohydrogenation of PUFA in the rumen, resulting in some form of protection being required. The objective of the current experiment was to manipulate the P:S ratio in lamb, whilst maintaining the *n-6/n-3* ratio, through feeding protected fat sources.

**Materials and Methods** Fifty Suffolk x mule lambs with an average liveweight of 29kg were individually penned and offered *ad-libitum* one of 5 diets based on barley and straw and formulated to contain 60g/kg DM fatty acids. The added fat sources were: Linseed oil, Fish oil, Protected Lipid Supplement (PLS; Rumentek, Australia), Algae/Fish oil (a mixture of marine algae (Martek, Colombia, USA) and fish oil in equal quantities on an oil basis) or Algae/PLS (a mixture of marine algae and PLS on an equal oil basis). The PLS was a spray dried emulsion of soya bean, linseed and sunflower oil treated with formaldehyde. The lambs were slaughtered at 40kg liveweight and muscle samples taken from the *longissimus dorsi* for fatty acid analysis by GLC. Results were analysed by ANOVA.

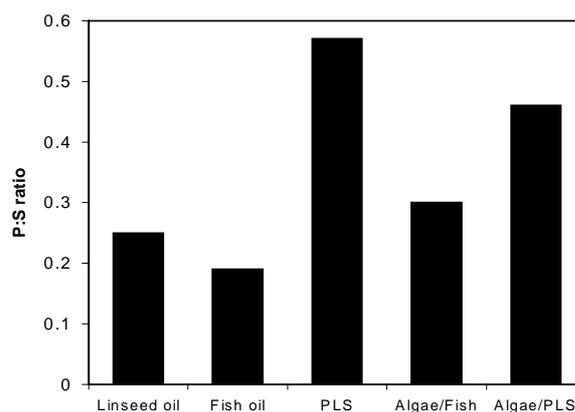
**Results** The total fatty acid, saturated fatty acid (SFA) and *cis-9, trans-11* conjugated linoleic acid (CLA) content of the *longissimus dorsi* was not significantly affected by treatment (Table 1). However, the content of *n-6* PUFA was highest in lambs fed the PLS diet whilst *n-3* PUFA and *trans-11* C18:1 was highest in lambs fed the Algae/Fish and Algae/PLS diets (P<0.001). Total PUFA content was lowest in the Fish oil and Linseed oil fed lambs and was approximately 2-3 times higher in lambs fed the PLS or Algae/PLS diets (P<0.001). The C18:2/C18:3 ratio was lowest in lambs fed the Linseed oil and highest in those fed the Algae/Fish oil (P<0.001; Figure 1). By contrast, the *n-6/n-3* fatty acid ratio was lowest in lambs fed the Algae/Fish oil diet at 0.68 (P<0.001). The lowest P:S ratio was obtained in lambs fed the Fish oil diet (0.19), and highest in lambs fed the Algae/PLS or PLS diets (0.46 and 0.57, respectively; P<0.001; Figure 2).

**Table 1** Effect of dietary fat source on the fatty acid content (mg/100g) of the *longissimus dorsi*

Fatty acid	Linseed oil	Fish oil	PLS	Algae/Fish oil	Algae/PLS	s.e.d.	Sign
<i>Trans-11</i> C18:1	158	177	108	175	217	21.5	***
CLA	36.2	26.8	26.4	28.4	33.1	5.39	NS
SFA	1376	1524	1446	1492	1516	172.5	NS
<i>n-6</i> PUFA	178	147	595	173	428	41.9	***
<i>n-3</i> PUFA	134	134	189	253	257	20.6	***
PUFA	312	231	784	427	686	60.4	***
Total fatty acids	3401	3706	3816	3673	3931	437.8	NS



**Figure 1.** C18:2/C18:3 and *n-6/n-3* ratios



**Figure 2.** P:S ratio

**Conclusions** Diet did not significantly alter the total fatty acid content of the *longissimus dorsi*. However, the P:S ratio in lambs fed the PLS diet was increased above the minimum recommended for the human diet as a whole. A mixture of PLS and marine algae resulted in a favourable P:S ratio of 0.46 whilst maintaining a nutritionally beneficial *n-6/n-3* ratio.

**Acknowledgements** We are grateful to DEFRA, ABN Ltd, Roche Products, Tesco Ltd, Pedigree Petfoods and the Rare Breed Survival Trust for supporting this work and Rumentek Industries, Parkside, Australia, for providing the PLS.

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# Increasing human intake of selenium by supplementing food animals with organic selenium

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**Introduction** The concentration of global crop and food animal production in regions where plant selenium content is low has led to a decline in the amount of selenium in the human food supply. The central reason is that where soil pH is acidic, selenium cannot be absorbed by plants, thereby preventing transfer of selenoamino acids up the food chain through cereal grains and food animal products. Direct addition of inorganic selenium salts prevents acute deficiency symptoms, however selenium salts added to food animal diets do not provide meaningful amounts of selenium in edible animal tissues. Because human selenium status is a public health concern, researchers have examined nutritional means of increasing the selenium content of meat, milk and eggs using selenium produced by yeast, which like higher plants are able to form selenoamino acids. While part of the focus is on producing 'designer' foods, a more general question pertains to both existing selenium levels in food animal products and to those when commercial food animals are given selenium in naturally-occurring organic vs inorganic form. The following summarizes selenium levels in edible tissues in commercial and controlled research settings where inorganic and organic (Sel-Plex<sup>TM</sup> selenium yeast, Alltech Inc.) were compared.

**Methods** All data were gathered under conditions where selenium content of feed ingredients was low (<0.1 ppm). Selenium sources were added at levels typically fed the herd or flock, with measurements of selenium content made in marketable products. Commercial trials with pig/poultry integrators were conducted over the normal growth to market age or weight timeframe and dairy trials were conducted over 3-6 months periods. Comparisons at 0.3 ppm Se are presented.

## Results

Where evaluated statistically, tissue selenium content was significantly increased by substitution of organic for inorganic selenium. Data from field summaries followed similar trends with magnitude of response often twice that noted in inorganic-supplemented diets, yet similar to tissue levels in regions where feed ingredients are rich in the element.

**Table 1** Effect of diet selenium form on selenium content (mg/kg) of animal products.

	Added diet Se	Inorganic Se	Organic Se <sup>1</sup>	Location	Reference
Pig meat (loin)	0.3 mg/kg	0.124	0.332 <sup>2</sup>	US	Mahan et al., 1999
	0.3 mg/kg	0.15	0.40 <sup>3</sup>	Australia	Henman, 2002 <sup>5</sup>
Broiler (breast)	0.3 mg/kg	0.160	0.373 <sup>3</sup>	Australia	Martin and Taylor, 2001
Milk	3 mg Se/d	<0.015	>0.030 <sup>2</sup>	Sweden	Ortman and Pehrson, 1997
	5 mg Se/d	0.015-0.020	0.030-0.040 <sup>3</sup>	Germany	Matzka, 1997
	4 mg Se/d	0.012-0.016	0.036-0.055 <sup>2</sup>	New Zealand	Knowles et al., 1999
Eggs	0.3 ppm	0.16	0.25 <sup>2</sup>	US	Paton et al., 2000
	0.3 ppm	0.044 <sup>4</sup>	0.243 <sup>2</sup>	US	Cantor et al., 2000
	0.3 ppm	0.659 (yolk)	0.711 (yolk) <sup>3</sup>	Britain	Kenyon, 2000

<sup>1</sup>Sel-Plex<sup>TM</sup>, Alltech Inc.; <sup>2</sup>P<0.05; <sup>3</sup>Commercial summary, not evaluated statistically; <sup>4</sup>Unsupplemented basal diet

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**Conclusions** Supplementing food animal diets with selenium from yeast has the potential to substantially increase human intake of selenium from meat, milk and eggs.

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## Relationship between linoleic and $\alpha$ -linolenic acids in cooked meat odour development

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**Introduction** During cooking, chemical reactions within the muscle produce volatile and non-volatile compounds characteristic of meat aroma and taste. Polyunsaturated fatty acids (PUFA) are essential in these reactions (Mottram and Edwards, 1983). In ruminants, differences in intramuscular PUFA composition have been classically associated with high n-3 content in grass fed animals and high n-6 content in concentrate fed animals. We investigated the effect on odour perception of *in vitro* reactions between linoleic and  $\alpha$ -linolenic acids in the presence of cysteine and ribose, when they were present in ratios similar to those found in meat from animals reared on forage- or concentrate-based diets.

**Material and methods** Six mixtures containing different ratios of linoleic and  $\alpha$ -linolenic acid, combined with a mixture of L-cysteine 0.5 mmol and D(-) ribose 0.5 mmol (Sigma Chemical Co.) in 0.2 M sodium pyrophosphate buffer (Elmore *et al.*, 2002), pH 5.5 were heated at 140 °C for 30 minutes. Each model system contained C18:2/C18:3 in a ratio of 2, 6 or 10 either with a fixed amount of linoleic acid of 0.51 mmol or with a fixed amount of  $\alpha$ -linolenic acid of 0.17 mmol. Four replicates were analysed from each model system. For sensory analysis using triangular tests, reaction mixtures were diluted 1/100 in buffer. Volatiles were trapped by Solid Phase Micro Extraction and analysed by gas chromatography/mass spectrometry (Elmore *et al.*, 2002).

**Results and discussion** At a fixed linoleic acid concentration increasing the amount of  $\alpha$ -linolenic acid produced highly significant differences between 2 vs 6 and 6 vs 10 ( $p < 0.001$ ) but no differences between ratios 10 and 2. When  $\alpha$ -linolenic acid was fixed differing in the amount of C18:2, no significant differences were found between any of the reaction mixtures with a very low number of positive identifications. This demonstrates that variations in the amount of C18:3 are the key to identification of mixtures with a different proportion of fatty acids and that differences in the amounts of linoleic acid have a smaller effect on odour perception. Some of the volatile results are shown in Table 2. Aldehydes derived from the oxidation of unsaturated fatty acids did not show significant differences between mixtures. Sulphur-containing compounds that have been reported to have roast meat-like, cabbage-like and onion-like odours were significantly altered in response to variations in C18:3. No differences were found when 18:3 was fixed.

**Conclusion** It is possible to create *in vitro* mixtures that imitate the reactions that happen during cooking in the muscle of animals fed different diets high in linoleic or  $\alpha$ -linolenic acids. Linoleic acid did not influence sensory perception as much as  $\alpha$ -linolenic acid. Variations in  $\alpha$ -linolenic acid content could be detected sensorially and have a greater effect on sensory perception.

**Table 1.** Number of correct answers in triangular tests of cooked mixtures of fatty acids (C18:2, C18:3) in a ratio of 2, 6 and 10, amino acid (cysteine, c) and sugar (ribose, r) when either C18:2 or C18:3 have been fixed.

	C18:2+c+r/C18:3+c+r		
	2 vs 6	6 vs 10	10 vs 2
18:2 fixed	15/17 ***	14/17 ***	7/17 n.s.
18:3 fixed	9/18 n.s.	7/18 n.s.	6/18 n.s.

**Table 2.** Percentage (std) of volatiles<sup>c</sup> of cooked mixtures of fatty acids (C18:2/C18:3) in a ratio of 2, 6 and 10, amino acid (cysteine) and sugar (ribose) when either C18:2 or C18:3 are fixed.

	18:2 fixed				18:3 fixed			
	2	6	10		2	6	10	
Aldehydes	15.2 (2.7)	11.9 (1.9)	14.9 (3.9)	n.s.	12.9 (2.2)	18.7 (2.3)	16.9 (6.0)	n.s.
S-containing	2.1 <sup>a</sup> (0.3)	1.5 <sup>b</sup> (0.1)	1.3 <sup>b</sup> (0.1)	**	1.8 (0.1)	1.5 (0.3)	1.5 (0.9)	n.s.

<sup>a,b</sup> Means with different superscripts on the same row are significant ( $p < 0.05$ )<sup>c</sup> Only compounds with more than 10 ng/100 ml have been considered. n.s. = no significant; \*\* =  $p < 0.01$ ; \*\*\* =  $p < 0.001$

**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 buffer feeding on milk fat composition from dairy cows offered a high-lipid ration at pasture

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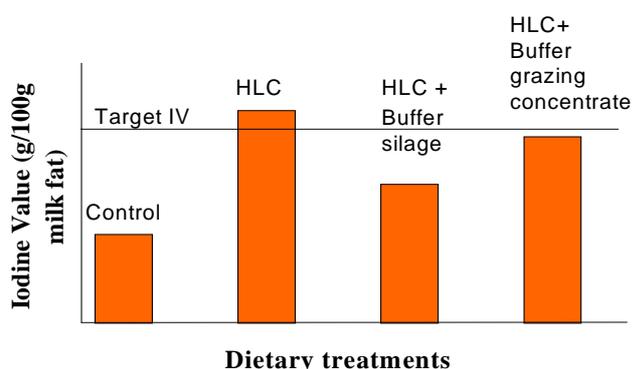
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**Introduction** Manipulation of the dairy cow's diet at pasture using high-lipid concentrate is an effective natural means of enhancing the composition and physical properties of milk fat and has been applied in several countries to improve the spreadability of butter. However, because of dietary energy deficits deriving from high yield potential of cows or grass of retarded or poor quality, dairy cows are frequently offered supplementary feeds or buffers e.g. silage or additional concentrates, while at pasture. The study investigated the effect on milk fat composition when providing dairy cows with buffer feeds while they received a diet of a high-lipid concentrate and grazed grass which aimed to increase the proportion of unsaturated fatty acids.

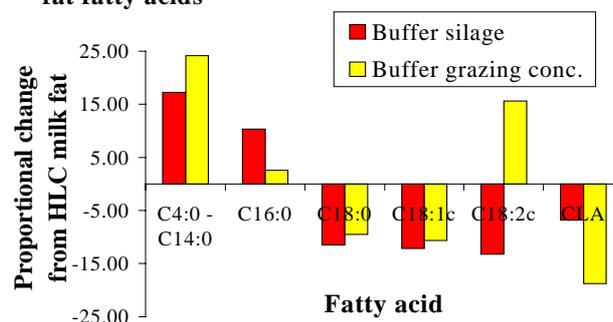
**Materials and method** Sixteen late lactation (mean days in milk 178) Holstein-Friesian dairy cows on summer grazing (24 h/day) were offered four dietary treatments. The control diet comprised 3 kg of a standard grazing concentrate (DM 873g/kg, CP 197g/kg DM); at pasture and was offered to four animals continuously over the six week trial period hence representing a dynamic baseline. The remaining twelve animals were randomly allocated within a 3x4 changeover design to one of three high-lipid concentrate (HLC) dietary treatments: 3 kg/day HLC (DM 870g/kg; CP 147.9g/kg DM; C16:0 111g/kg, C18:1 498g/kg, C18:2 245g/kg, C18:3 91.2g/kg); 3kg/day HLC with grass silage (DM 309g/kg) offered *ad libitum* post milking morning and evening; 3kg/day HLC with 4.6kg/day standard grazing concentrate. The fat source in the HLC was supplied by whole rapeseed (C16:0 41g/kg, C18:0 18g/kg, C18:1 609g/kg, C18:2 210g/kg, C18:3 88g/kg). All concentrates were pelleted and the daily allowance was offered as equal quantities at the morning and evening milkings. Milk samples were collected from individual animals twice weekly. Milk fat was extracted by chloroform:methanol (2:1 v/v) and analysed for iodine value (IV) by Wij's method (AOCS, 1990) and fatty acid methyl esters (FAME) by gas chromatography (Fearon *et al.*, 1994).

**Results and discussion** Fig 1 depicts the mean IVs of the milk fats from each dietary treatment compared to the target IV. IV is a measure of the degree of unsaturation of a fat, a higher IV reflecting a higher content of unsaturated fatty acids. The target IV was the minimum required to make the spreadable butter product. The HLC diet, resulted in the highest IV, milk fat IV from the HLC + buffer silage diet was however considerably below the target IV. Similarly, inclusion of buffer grazing concentrate to the HLC diet also had a detrimental effect on IV, although to a lesser extent. These results are supported (Fig 2) by the proportional change in fatty acid composition as a result of the dietary treatments. Overall the saturated fatty acid content has increased and the unsaturated content decreased as a result to buffer feeds with silage buffer having a more marked effect. It is interesting to note, however, that the grazing concentrate decreased the milk fat CLA content to a much greater extent than the silage buffer. The buffer feeding may be providing a dilution factor in the diet by supplying increased volumes of saturation, therefore diluting the unsaturation being supplied by the high-lipid concentrate. Alternatively, the more fibrous silage and differing composition of concentrate may have influenced the rumen microbial profile, which would therefore alter the output from the rumen and hence affect the milk fat fatty acid profile.

**Fig. 1 Effect of buffer feeding on milk fat IVs**



**Fig. 2 Effect of buffer feeding on major milk fat fatty acids**



**Conclusion** The results of the present study demonstrate that buffer feeding in the form of either grass silage or grazing concentrate, to dairy cows at pasture and offered a high-lipid supplement had a detrimental effect on milk fat compositional properties as measured by IV and fatty acid analysis. The inclusion of buffer silage had a greater effect than the grazing concentrate buffer thereby producing a less spreadable butter.

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## The cholesterol level in *m.longissimus dorsi* of pigs according to the type of cross and different body weight at slaughter

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**Introduction** Consumers want meat with low levels of fat and cholesterol. The fatty acid and cholesterol content of pork could be influenced by nutritional and genetic factors, and cholesterol content can also be affected by slaughter weight (Barowicz and Pietras, 1988). This study therefore examined the cholesterol content of pork from three commercial pig breed crosses slaughtered at three live weights.

**Materials and methods** The study were carried out on crossbred fatteners originated from cross between LY sows (Landrace x White Large) and different boars – Duroc, Hampshire and Pietrain breed.

Fatteners from 30 kg of body weight till slaughter were feed *ad libitum* by the same feed with following composition; 13,21% of protein, 13,09 MJ of energy, 6.5% of crude fat, 0,9% of lysine, 0,29% of methionine, 0,18% of tryptophan, 0,57% of threonine and 0,83% of calcium. The ingredients used in diets – wheat, barley, soyabean, meat meal and 5% premix. The fatteners were slaughtered at 95, 105 and 115 kg of body weight. In each weight group were 18 fatteners – 6 from each cross. The samples of meat were taken from the *m.longissimus dorsi* between 4 and 5 lumbar vertebra and content of cholesterol was analysed according to Rhee et al., (1982) method. Statistical differences were tested by two-way Analysis of Variance.

**Results** The cholesterol level in different cross and different body weights are given in Table 1.

There were no statistically significant differences in cholesterol level depending from cross and body weight.

**Table 1.** The cholesterol level (mg/100g of tissue) in *m. longissimus dorsi* according to cross type and body weight

	M. longissimus dorsi
	$\bar{x} \pm SE$
N	54
<hr/>	
LYD	
95 kg	46.98 ± 3.78
105 kg	39.28 ± 4.09
115 kg	43.21 ± 2.97
LYH	
95 kg	46.50 ± 3.66
105 kg	40.25 ± 2.97
115 kg	44.20 ± 2.84
LYP	
95 kg	39.91 ± 3.97
105 kg	42.65 ± 3.29
115 kg	45.68 ± 3.16

**Conclusion** The result of the current study demonstrate no statistically significant differences in cholesterol level in tissues from three commercial pig breed crosses but also no influence of body weight at slaughter. However, crossbred fatteners with Duroc blood had the lowest and fatteners with Pietrain blood had the highest level of cholesterol when slaughter at 115 kg which is a normal slaughter weigh in Poland.

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## **Disease and Biosecurity**

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There are large differences between the prices of animal products in regions with comparative advantage in livestock production and regions where production costs are higher. In particular, prices for many animal products are much higher in The European Union (EU) and The United States of America (USA) than in regions with extensive grazing areas or low-priced animal feed grains, e.g. Australia, South America and Southern Africa. These price differentials provide strong financial incentives for trade in animal products between these regions. However, trade is constrained by three main factors:

1. The perishability of many animal products, resulting in additional processing costs (e.g. freezing) which may also reduce the value of the product and higher transport costs.
2. The fact that in many markets, high prices have been used as an element of agricultural support policy. These are partly maintained by levies on imported products.
3. Zoosanitary restrictions to control the risk of introducing livestock and human disease agents in animal products.

The first two constraints have become less significant in recent years as a result of improved processing techniques, lower transport costs and international initiatives to liberalise world markets and stimulate international trade. However, zoosanitary controls remain as an important barrier to trade in livestock products. In some cases, they make trade impossible, and in others they increase costs to exporters (and therefore prices to importers) often to the point where trade is uneconomic.

Various risk assessment and management techniques have been used to minimise the impact of zoosanitary measures on international trade in animal products. These include international standards for assessment of the animal health status of countries and regions, and the use of risk assessment techniques to balance the disease risk of trade and the cost of zoosanitary measures. The value and credibility of these methods is limited by the difficulty of providing estimates of the probability of trade in a particular commodity resulting in the transmission of a disease agent, and of the economic loss that would result. These methods have been useful in comparing the relative efficiency of alternative zoosanitary controls, but less successful in providing objective estimates of absolute risk. The subjective judgements often required in exotic disease risk assessment mean that there may be no basis to determine whether restriction or prohibition of trade is justified.

It is also the case that the interests of veterinary authorities in importing countries would rarely be advanced by accepting any risk or uncertainty. They bear the responsibility for any failure to prevent the introduction of exotic disease agents, and gain no advantage from promoting trade. On the contrary, they may be under pressure from protectionist lobbies to restrict importation.

# Knowledge transfer and dissemination: insights from the dairy sector in the UK and India

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**Introduction** ‘Knowledge transfer’ and ‘uptake’ are high on the agenda of government departments and research institutes. In the U.K., the Department for Environment, Food and Rural Affairs (DEFRA) is concerned that new knowledge generated by publicly funded research is not being taken up by the industry and has funded a series of knowledge transfer initiatives. On the international front, the Department for International Development (DFID) is pressing managers of its various research programmes to demonstrate that the outputs of research are being used by farmers in developing countries and are having an impact on their livelihoods. International institutes are increasing the proportion of their resources devoted to technology transfer and dissemination programmes. These concerns and initiatives raise important conceptual, institutional and methodological issues.

**Knowledge transfer** At the level of the farming industry, the concept of knowledge being transferred from research to its application seems straightforward. At the level of the individual who must decide whether or not to use new ideas and technology, the concept is much more problematic. Knowledge ranges from simple awareness of a new technology, to what one believes about its efficacy and usefulness. Transferring the latter is less straightforward than the former. Knowledge is personal. One person cannot transfer their knowledge to another. We can tell people about new research findings and how these can be incorporated into their farming practice. But what they end up “knowing” about them may be very different from what we thought we were “transferring”. We can pass on information; the effect that this has on the knowledge of an individual is influenced by what he or she knows already, their evaluation of the information and their perceptions of those who are doing the transferring. In the UK dairy sector, scientists know that fertility rates are declining, that this is partly due to some heats going undetected, and that there are technologies which have been proved to improve heat detection<sup>1</sup> but have not been widely adopted by farmers. A recent (2002) survey of a random sample of 500 dairy farmers in south-west England shows that many farmers do not think they have a problem with heat detection, evaluate the new technologies as not cost-effective particularly with milk prices at their current levels and think that their own experience, stockmanship and record keeping are the most reliable aids to heat detection. This highlights that knowledge includes a strong subjective element which is used to evaluate new information. Current knowledge can impede the acceptance of new ideas. A 2002 survey of 890 smallscale dairy enterprises in peri-urban areas around Pondicherry, India, identified current knowledge about symptoms, causes and treatment of ten common diseases and conditions of dairy cattle<sup>2</sup>. In response to an open question, cattle owners mentioned 38 causes of repeat breeding, most of which would not be regarded as correct by veterinary scientists, while one third of respondents were not able to identify any cause.

**Institutions and methods for knowledge transfer** Farmers’ prefer personal and interactive means for accessing information and advice, including itinerant milkers (India), other farmers and vets (India and UK). A recent study for DEFRA<sup>3</sup> reviewed arrangements for providing advice to land-based enterprises in Europe, North America and Australasia, in the light of concerns that privatisation of government advisory services has contributed to fragmentation and inefficiencies. This study concluded that farmers benefit from a diversity of information and advisory services and that governments should not seek to over-manage the provision of advice.

**Conclusion** Any strategy for knowledge transfer and dissemination of research outputs must take account of what farmers currently know, including their attitudes and perceptions, and of how they access and evaluate information and advice. Top-down models of technology transfer should be tempered with interaction and dialogue.

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## **Sustainable breeding objectives in developing countries**

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In the developed world, the last fifty years has seen a great convergence of breeding objectives and strategies in all farmed species. This is part of the effects of globalisation, which has led to ever increasing specialisation of livestock producers. A general consequence is that breeding goals and structures of dairy, beef, pig and poultry production are now very similar throughout the developed world.

After some decades of successful concentration on narrow breeding goals (yield of milk solids in the dairy sector, growth, feed conversion and lean yield in meat animals) objectives have now broadened to take in product quality, reproduction and disease traits.

In developing countries, pig and poultry production systems now frequently follow the same path. However, the poorer the country, the more likely it is that production is predominantly in traditional ruminant systems, reliant on seasonally variable feed supply, and subject to severe disease and climatic challenge. In their breeding objectives and structures, each of these situations has its own opportunities, constraints and therefore requirements. The paper illustrates a range of case studies. A general framework is given, against which these can be evaluated.

## **GM technology: a tool to benefit livestock production in less developed and developed countries**

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**Introduction** The combined effects of improved varieties, increased fertiliser use and irrigation coupled with increased pesticide use was instrumental in allowing world food production to double in the last 35 years. However, as we enter the 21<sup>st</sup> century world population is set to increase by a further 1.5 billion by 2020, with the rate of increase being higher in less developed than developed countries. At the same time the rate crop improvement is slowing. In addition the area of land available/appropriate for the production of food and feed resources are at best static and at worst decreasing, often due to wind and water erosion. It has also been estimated that the demand for livestock products will increase dramatically in the next 20 years, with the increase being markedly higher in developing countries (3% per year), where much of the increased demand will be generated from an increasing urban population, than developed countries (1% per year). This projected increase in demand for livestock products will require very significant increased production of cereal grain and sources of oilseeds in a manner ensuring safety for the public and the environment. It will be a major challenge for global food and feed production to decrease the environmental impact of agriculture while maintaining or improving its productivity and sustainability. This paper will discuss how GM technology can contribute a way forward with the aim of combining higher yields, improved food and feed quality, increased competitiveness with environmentally and agriculturally sustainable practices.

**Uptake of GM Technology** It is reported that in the year 2002, 6 million farmers in 16 countries grew 59 million ha of transgenic crops. The increase from 4 million ha in 1996 to nearly 59 million ha in 2002 represents the fastest adoption rate of any new technology ever brought to agriculture with an accumulative total of 235 million ha grown in this period. These crops (First Generation) have in general been modified for agronomic input traits such as herbicide tolerance and/or insect protection, while the next generation of crops, which are currently in field trials, have modified for improved nutritional characteristics. While in 2002 approximately 75% of the area is grown in developed agricultural systems, 25% is now being grown in developing countries and there is clear evidence that it scale neutral as shown by the success of the smallholder schemes in both South Africa and India.

**Limitations to livestock production** The limitations are numerous and varied but are applicable to a greater or lesser extent in both developed and developing countries. Crop factors that limit livestock production include inadequate quality (e.g. crop residues such as maize stover) and quantity of feed resources with an inconsistent supply due to extremes of climate (e.g. low rainfall and high temperatures), the presence of anti-nutritional factors and toxins (e.g. mycotoxins), and deficiencies of specific nutrients (e.g. amino acids and mineral). While improved animal genetics have greatly increased productivity/animal (e.g. high genetic merit cows) in developed countries, the low genetic potential of many of the breeds in developing countries is a limiting factor. However, improved feed resources are usually needed before increased animal productivity can be achieved through enhanced genetics. Furthermore the occurrence of animal diseases is a serious limitation in livestock production.

**Environmentally and sustainable agricultural practices** Studies have established that growing agronomic input trait GM crops reduce pesticide inputs in crops that often form the cornerstone of ruminant and monogastric livestock production. In addition these crops are sprayed less frequently, the consequences of which are a significant reduction in diesel use and emissions of CO<sub>2</sub>. For example in Argentina the reduction in field operations associated with GM crops has saved 100 million litres of diesel and reduced CO<sub>2</sub> emissions by 350,000t. The reduced input costs and the often-associated increase in yield enhances economic competitiveness. Second generation GM crops will also provide significant environmental benefits. For example crops modified for improved nitrogen digestibility or those with high phytase or low phytate content will be a major factor in reducing nitrogen and phosphorous excretion. Products that can manipulate rumen fermentation will also be developed resulting in reduced methane production. The introduction of GM technology has resulted in a large and significant increase in the use of conservation tillage with associated reductions in wind and water erosion and further reductions in the use of diesel and hence emissions of greenhouse gasses.

**Safer feed and improved animal performance** In many parts of the world maize is attacked by a variety of insect pests, for example the European Corn Borer that can significantly reduce crop yield and decrease the quantity of food and feed available for both humans and livestock. After the initial insect attack the crop is susceptible to secondary fungal infection as the spores enter the wounds left by the insect. Modern biotechnology has produced commercialised insect-protected plants that have been genetically enhanced to produce proteins similar to those produced by the soil bacterium *Bacillus thuringiensis* (*Bt*). Research has demonstrated that while crop yields can be enhanced there is a consistent and significant reduction in total fumonisin (B1, B2 and B3) concentrations in maize kernels containing the Cry1Ab protein when the hybrids were infested with European Corn Borer. A safer maize supply will result in reduced incidence of mycotoxicosis (fumonisin) in livestock and increased animal performance as demonstrated by studies with pigs and poultry.

**Alleviation of abiotic stresses** Many millions of the poorest people in the world live in marginal areas subjected to low and erratic rainfall, extremes of soils conditions, extremes of temperatures and salinity. Work is in progress to modify plants to withstand these abiotic stresses. One example is plant modification to withstand aluminium in acid soils

**Improved nutrient density** Increasing nutrient density of livestock diets can improve both productivity and efficiency. For example in many parts of the world where crop residues are a major forage source forages will be modified to contain more digestible fibre components and/or fibre digestive enzymes and rumen bacteria will be developed which will improve efficiency of fibre digestion. Such improvements would also be very pertinent to livestock production systems in developing countries as fibre digestion of most crops by ruminants is far from optimum. In addition energy density of animal feed resources can be enhanced by increased non-structural carbohydrates such as starch and oil content.

**Enhanced nutrient content** Plants are an important protein source for both human and animals. While cereal crops such as maize, rice, and wheat contain between 7 and 14% protein, legume crops can contain up to 50% protein. However, most plant proteins are deficient in at least one essential amino acid, with cereal proteins being generally low in lysine content and legumes being low in sulfur containing amino acids such as methionine and cysteine. Supplementation of specific amino acids is often needed to avoid deficiencies in non-ruminant diets. Modern biotechnology will aim to improve not only protein content but also the amino acid profile of a wide range of crops.

**Improved quality of animal products resulting from the use of GM feed ingredients** Approximately 20% of total energy intake in industrialized countries and as knowledge improves as to their related health effects more emphasis is being placed on the quality of oils and fats in the human diet. Thus the degree of saturation of fats, fatty acid composition, and stereo configuration of individual fatty acids are all targets for genetic modification. These modifications are being implemented in a number of crops including but not limited to soyabean, maize, canola, cotton, and sunflower. For example high oleic acid soyabeans can contain more than 80% oleic acid in their oil compared with 24% for traditional soyabean oil and research has indicated that feeding high oleic full fat soya to cows and chickens may result in a lowering of the saturated fat levels in milk and poultry meat. In addition work is in progress meat quality and lessen the danger of *E. coli* outbreaks when meat is contaminated during slaughter and processing. It is now possible through modern biotechnology to make specific antibodies to be produced in crops such as soyabeans which could be fed to livestock prior to slaughter to reduce or eliminate outbreaks of food-borne diseases such as *E. coli* and *Salmonella*.

**Improved animal health** In both developed and developing countries both ruminants and monogastrics are susceptible to a wide range of diseases that are responsible for significant reduction in output of milk, meat and eggs. Disease prevention often entails vaccination that is often costly and difficult to achieve effectively and efficiently. Crops are being modified to act as an edible vaccine that can be stored and distributed as seeds, tubers, or fruits. For example plants have been modified to produce vaccines against a variety of animal diseases such as Rhinovirus, Foot & Mouth and avian influenza.

**Conclusions** The paper illustrates how the introduction of first generation GM crops modified for agronomic input traits such as herbicide tolerance and insect protection has resulted in significant environmental benefits that will help in the development of more sustainable agricultural production systems, through the reduction of inputs such as pesticides and diesel. In addition it has shown how feed resources are being modified for improved nutritional characteristics which will offer significant advantages to livestock producers not only in terms of increased production and improved efficiency but also in providing food products with improved quality and nutritional characteristics.

## **GM technologies – opportunities and threats of applying GM technology in less developed and developed countries**

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The Food and Agriculture Organisation (FAO) and the Consultative Group on International Agricultural Research (CGIAR) confidently predict that the demand for meat will increase by 50 percent between 2000 and 2020 and for eggs by 25 percent over the same period. They predict that the growth in demand will be greatest for pigs and poultry products. This challenge will be met through increases in production and productivity.

Increases in demand will be driven a combination of population increase and economic growth. The greatest increases will be in the rapidly growing economies of Asia – particularly in India and China. While local production meet much of the growing and diversifying needs of consumers it is also inevitable that international trade in animal products will grow. As the wealth of consumers increase so will their tastes diversify together with their interest in sources of food and choice. BSE and outbreaks of foot and mouth have focused consumer interests on food safety issues and have in part contributed to concerns over the production and use of genetically modified organisms.

Possible changes in production subsidies as a result of a successful outcome to the WTO Doha Round could have a major impact on global and local markets and patterns of supply in developed and developing countries.

The challenge of meeting the growing demand for livestock products will also have to be achieved in the context of increasing demands for a wide range of economic, social and environmental services derived from our natural resources and the way they are managed. It will have to be done against a background of growing competition for land, water, environmental protection, the impact of HIV/AIDs and possible climate change.

Improvements in animal health, welfare, nutrition, management and productivity will be necessary in meeting demands and in trade practices. Advances in genomics and biotechnology have opened either an Aladdin's Cave or Pandora's Box of opportunities and tools. Vaccines and diagnostics can play a vital role in improving animal health. Transgenic animals and plants could synthesise an increasing range of proteins and other metabolites with pharmaceutical and industrial value. GM technologies could increase yields, impart drought tolerance and pest and disease resistance and improve the nutritional value of animal feeds and fodder. They could also reduce the presence of pesticide residues in food and water supplies.

However this potential will only be realised if GM technologies are accepted by society as safe and reliable and that they are responsibly used. There are those have raised the spectres of gene flow, super bugs, allelgenicity and long term impacts on health and the environment. These are concerns cannot be ignored , they must be unaddressed even if some of them may prove unfounded.

This will require considerably more investment in risk assessment, regulation and the capacity to manage the processes to acceptable standards. It will also require greater international acceptance and trust of regulatory systems and standards. The costs of getting GM technologies into the market place are already higher than more traditional technologies; for many developing countries these costs and capacities needed are beyond their means. The fact that many of these technologies are emerging from the private sector means they are inspired by market opportunities, but public sector funding has not kept pace and this has created tensions, intellectual property issues and reduction in the availability of GM technologies and risk assessment tools as public goods.

To beat the challenges of rising demand while addressing the problems of poverty, environmental change and instability will require a wide range of skills, technologies and partnerships. Current discord over the use of some technologies and mistrust of the private sector makes constructive discussion difficult. The solution rests in all sides taking risks, build trust and focusing on the real challenge to stability and sustainability – reducing poverty.

## Introduction to functional foods

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It is axiomatic that adequate supplies of good quality food is beneficial for health. Conversely it has also long been recognised that populations suffering from malnutrition are more susceptible to various diseases and have poor health. However the concept of functional foods, as foods that offer some health-associated advantage over conventional foodstuffs is a relatively recent innovation into the human nutrition market. Functional foods are generally defined as foods that have an effect on well-being and health or result in reduction in disease risk. The advantages offered by functional foods are generally related to disease avoidance and health maintenance rather than to therapeutic effects of foods. Functional foods are now part of a worldwide nutrition market estimated at \$156 billion in 2001 (Starling, 2002). However, they can only become significant in societies where food security is assured and basic foodstuffs are relatively cheap. Consequently the major markets are North America, Europe and Japan.

**Nature of functional foods** They must offer some advantage related to disease avoidance and health maintenance. They have been implicated in alleviating the risk of a whole range of non-infectious diseases and have an impact upon immune status and viral mutation. There are now functional foods targeted at skin, gut, heart, joint, eye, and cognitive or mental health. Most functional foods are vehicles to deliver some particular bioactive components or nutraceuticals (Adams, 1999) in the food which have a beneficial effect upon health. Many nutraceuticals are of plant origin and currently the vast majority of functional foods are based on molecules of plant origin (Amadó *et al*, 2002). These include carotenoids, flavonoids, non-digestible oligosaccharides, organic acids, phospholipids, and polyphenols. Functional foods of animal origin are not so prominent as animal products do not contain the enormous range of secondary metabolites characteristic of plants. However foods of animal origin do have several useful nutraceuticals such as, lutein in eggs, various carotenoids and long chain polyunsaturated fatty acids in fish, conjugated linoleic acid (CLA) in milk. Other valuable nutraceuticals in animal source foods are collagen, chondroitin, taurine, selenium, butyric and lactic acids. A further advantage of animal source foods, particularly meat is the high content and bioavailability of micronutrients such as iron, zinc, and vitamin A from meat and vitamin B<sub>12</sub>, riboflavin, and calcium from milk.

**Dairy products as functional foods** Dairy products may protect individuals against insulin resistance syndrome which are major risk factors for diabetes and heart disease (Pereira, 2002). An active area of development of functional foods is the fortification of yoghurts with probiotic cultures or with non-digestible oligosaccharides. Many functional foods are supplemented with calcium and there is now an increasing trend in using milk calcium as a source of calcium fortification. Conjugated linoleic acid (CLA) is a component of milk fat beneficial to human health. Lactose, stimulates the absorption of calcium from the gastrointestinal tract. Enzymatic digestion of milk proteins releases various nutraceutical peptides which facilitate nutrient uptake, influence immunoregulation and ensure optimum functioning of the gastrointestinal tract and thereby avoid disease. Important nutraceuticals recognised in milk are: lactoferrin,- an iron-binding protein, lactoperoxidase,- an antibacterial enzyme system and angiotensin-converting enzyme (ACE) inhibitors, which reduce blood pressure and reduce the risk of cardiac diseases and strokes.

**Meat as functional foods** Meat is a valuable source of highly bioavailable nutrients but has been criticised on the basis of its saturated fat content. However diet has a pronounced effect upon the fatty acid composition of muscle tissue and meat quality can be modified in favour of more unsaturated fatty acids. Indeed modern meats frequently have quite low fat contents. Meat also has an important synergistic effect with foods of plant origin and the presence of meat in a diet enhances the absorption of zinc and iron from cereal and other plant sources (Neumann *et al*, 2002). Foods of animal origin supply high-quality and readily digested protein and energy and are also a compact and efficient source of readily available micronutrients. Consequently foods of animal origin are to a large extent already functional foods and this needs wider recognition.

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## Animal fats and human health

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In 1991 it was recommended that total fat intake in the UK should be reduced to a population average of less than 33% of total daily energy intake and that saturated fatty acids should contribute no more than 10% of total energy (Department of Health, 1991). A further recommendation was that the intake of *trans* fatty acids should not exceed 2% of total energy. These recommendations were made primarily on the basis of the influence of fatty acids on plasma cholesterol and thereby on the development of cardiovascular disease. While associations of fat intake with other chronic diseases such as cancer, obesity and diabetes have also been suggested, it was felt that there was insufficient evidence to make specific recommendations on the basis of such claims. A reduction in saturated fat intake has remained a central target of public health nutrition within the United Kingdom ever since. Despite concerted efforts, particularly throughout the 1990s, to achieve these targets little progress has been made. In 2000, total fat intake remained at 38% and saturated fatty acid intake at 15% (DEFRA, 2001).

Animal products represent a major source of dietary saturated fatty acids. The National Food Survey showed that 22% of dietary saturated fatty acids were obtained from meat and meat products and 39% from dairy produce (DEFRA, 2001). In recent years considerable effort has been put into trying to increase the unsaturated fatty acid profile of meat and dairy produce (Salter *et al*, 2002). This poses particular problems in ruminants where dietary unsaturated fatty acids are largely hydrogenated in the rumen. However, a variety of strategies have been developed to protect the fatty acids from hydrogenation and significant increases of both n-6 and n-3 polyunsaturated fatty acid content of ruminant meat and milk have been achieved. The relevance of these changes, set against the relatively high saturated fatty acid content of these foods, remains controversial.

The recommendation for maintaining a low level of *trans* fatty acid in the human diet is primarily based on data suggesting that elaidic acid (C18: 1, *trans* 9) raises plasma cholesterol concentrations (Department of Health, 2001). Most of the elaidic acid in the human diet arises from the hydrogenation of vegetable oils to produce margarines and shortenings. Animal products, particularly those from ruminant animals, contain a range of other fatty acids containing *trans* double bonds. These represent intermediates of rumen hydrogenation of unsaturated fatty acids, which escape the rumen and enter the tissues. One class of fatty acids containing *trans* bonds are the conjugated linoleic acids (CLAs). This group of positional and geometrical isomers of linoleic acid (C18:2) have been ascribed a range of potential health benefits. The best established are an anti-carcinogenic effect and a reduction in adipose tissue deposition. The most abundant isomer occurring in ruminant tissue is *cis* 9, *trans* 11 which may be specifically associated with protection from cancer. It has recently been demonstrated that through a combination of genetic selection and dietary manipulation the concentration of this isomer in cow's milk can be significantly increased (Bauman *et al*, 2000). However, this increase is also associated with an increase in the amount of the monounsaturated fatty acid, vaccenic acid (C18:1 *trans* 11) in the milk. Little is known about the impact of vaccenic acid (as opposed to elaidic acid) on human health. However, recent evidence suggests that we may be able to convert this into *cis* 9, *trans* 11 CLA (Adlof *et al*, 2000). Thus, the effects of specific *trans* isomers of fatty acids on human health require further investigation.

In summary, despite concerted efforts to reduce the saturated fatty acid intake in the UK, little progress has been made toward the targets set. Further research into reducing the saturated fatty acid content of animal products, particularly those from ruminant animals, is required. The health benefits of CLAs to humans remain to be established but there is an urgent need to clarify the effects of specific isomers of fatty acids that contain *trans* double bonds.

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## Enhancing the selenium content of food products from animals

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Selenium (Se) was first recognised as an essential nutrient in 1957. Subsequent research has shown that Se, in the form of selenocysteine, is incorporated into a range of selenoproteins in the body, which are crucial to human and animal health. The best known of these is glutathione peroxidase (GPx), which is involved in protecting cell membranes from oxidative damage by free radicals. Other selenoproteins are essential in the functioning of the immune system, in thyroid hormone metabolism and in reproduction.

The UK Reference Nutrient Index (RNI) recommends daily intakes of 60 µg for women and 70 µg for men. In contrast, Se intake in the UK has declined over recent years, from 60 µg day<sup>-1</sup> in 1974 to 39 µg day<sup>-1</sup> in 1997 (MAFF, 1997). This decline has been attributed to the increasing use of European wheat in preference to Se-rich North American wheat, together with a decline in household consumption of cereals, particularly wheat. While estimated intakes from the MAFF Food Surveillance study may not be directly comparable with reference nutrient intakes, there is concern that intakes are at the lower end of the recommended reference range for adults.

Should we be concerned about the discrepancy between intake and RNI? While diseases in both humans and livestock associated with acute deficiency have been widely reported, research has suggested that less overt deficiency can adversely affect disease susceptibility and maintenance of health. Selenium deficiency has been associated with a loss of immunocompetence, thyroid function, cardiovascular disease, and the occurrence and development of viral infections (including HIV) and certain cancers (see Rayman, 2000). Se appears to be essential for brain function, and in a number of studies Se deprivation has been associated with increased feelings of anxiety, depression and fatigue while supplementation has been reported to improve mood.

If the shortfall is to be remedied by dietary changes, a number of strategies may be considered. In Finland, where the Se contents of soil are particularly low, augmentation of fertilisers with Se has proved an effective strategy, and resulted in three-fold increases in the Se contents in bread, milk and meat (Vario *et al.* 1994). Uptake of Se from the soil is, however, variable and influenced by crop, soil type and climate. Sulphur, which is being increasingly added to fertilisers to increase the efficiency of nitrogen utilisation by plants and plant growth, also reduces Se uptake by plants.

An alternative – or complementary – approach is to enhance the Se content of animal products. There is now considerable evidence to confirm that appropriate supplementation with Se can increase the Se content of eggs, meat and milk. However, the effectiveness of this approach depends on the form in which the Se is given. Se in feed is predominantly present as selenomethionine (plant and animal sources) or selenocysteine (mainly animal products). Under current legislation, only inorganic Se (selenite, selenate) may be used to supplement livestock diets. The active form of Se in selenoproteins is selenocysteine; selenomethionine may be incorporated into proteins in place of methionine, where it acts as a reserve and becomes available with normal body protein turnover. In contrast, only a relatively small amount of inorganic selenium finds its way into body proteins. While some of the absorbed inorganic Se is reduced to selenide to provide selenophosphate, the precursor to selenocysteine, inorganic Se not used for selenoprotein synthesis is transported to the kidney and excreted in urine. In ruminants a significant proportion of dietary inorganic Se is reduced by rumen micro-organisms to insoluble and unavailable forms, which are excreted in the faeces. As a result, the ability to substantially increase the Se content of animal by supplementation with inorganic Se, while keeping within regulatory maximum inclusion levels, appears to be limited. For this reason, there is increasing interest in the use of organic Se in the form of Se-enriched yeast (e.g. Sel-Plex, Alltech, Inc.) where the Se is predominantly in the form of selenomethionine. A number of studies have confirmed the advantages of this product over inorganic forms of Se in increasing the Se content of animal products, and an application has recently been made to the EC for approval of Sel-Plex as a supplement for livestock in Europe.

It is important to register a note of caution when advocating supplementation with Se. The toxicity of Se was well established long before it was identified as an essential element. Although authorities differ in estimates of tolerable upper intake levels, the EC Scientific Committee on Food recently recommended a level of 300 µg day<sup>-1</sup> from all sources, including supplements. It appears therefore that there is scope for increasing Se content of food products from farm livestock, and thus the Se intake by the human population, while remaining within tolerable intake levels.

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## Genetic disorders in dogs

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There has been a shift over the last few years in the profile of diseases that veterinarians encounter in the dog. Improvements and developments in antibiotics, antihelminthics and more effective vaccines have controlled many of the infectious diseases that have caused problems in the past. As a result, there has been a relative increase in diseases that have a genetic basis.

In excess of 350 inherited diseases, or diseases where inheritance is thought to play a part, have now been recognised in the dog. Of these, we know the precise mode of inheritance in approximately 55% of cases, and of these approximately 70% (around 140 distinct conditions) are the result of a single recessive mutant gene. Of the rest, a small percentage result from a single dominant mutation and the rest represent complex or polygenic conditions, where more than one gene requires to be mutated, usually in a recessive fashion, in an affected dog. Unfortunately, many of the inherited diseases that exercise breeders' minds the most, like hip dysplasia, epilepsy auto-immune diseases, heart disease and cancer, are polygenic in nature.

One of the major challenges facing present-day dog breeding is to address, control and eradicate these inherited diseases. Unfortunately, as the figures above show, the vast majority results from recessive mutations and this makes control much more difficult. This is because clinically normal dogs can in fact be carriers of the mutation. Although carriers will not become clinically affected, and are consequently therefore very difficult to identify, they will pass on a mutant version of a disease-causing gene to some of their offspring. In this way, carriers act like silent genetic reservoirs of the mutation dripping mutant genes into the gene pool causing the breed frequency to increase. Ultimately a point will be reached where the probability that two carriers will mate and produce one or more affected offspring becomes high.

Identifying carriers before mating is key to a breeder's efforts to control the spread of inherited disease. At the moment, the only way that carriers can be identified for most of these conditions is to use traditional pedigree analysis. Take a condition that results from a single recessive mutant gene, like Progressive Retinal Atrophy in the Labrador. If a litter is born to clinically normal parents having one or more affected puppies, then both parents are obligate carriers. Unfortunately, this kind of analysis can miss a number of carriers, for example, the carrier frequency may be low so that the chances of two carriers mating to produce an affected offspring will be low. Similarly, two carriers could mate but, by chance they don't actually produce an affected puppy.

If progress is to be made we need a more reliable method of identifying carriers so that breeders can take this into account when developing their breeding programmes. Fortunately, recent progress of the canine genome project has provided detailed genetic maps of canine chromosomes which will help scientists to identify the genetic mutations that cause these inherited diseases. Once a mutant gene has been identified, a simple DNA test can be developed to identify the presence of a mutant gene in an individual dog. Such developments greatly add to the armoury that a breeder can use to address inherited disease. By testing all potential breeding stock before they are mated a breeder will know whether they have a genetically clear dog, a carrier or, perhaps an affected, and take note of this when deciding whether to breed from the tested dog or not.

## **Non-domesticated animals as pets**

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Humans have kept animals for companionship for thousands of years. Developments during the twentieth century in intercontinental transport, especially air travel, in the husbandry of animals, and in the technology for their housing; combined with widespread availability of electricity to power environment control systems (water filtration, oxygenation, heat lamps, humidifiers, photoperiod control etc), have made it possible to obtain and maintain a very much wider range of species of wild animals in captivity than ever before. Associated with this, there has been a huge increase in interest in keeping ornamental fish, amphibians, and reptiles, and the range of species of birds and mammals kept as pets has increased markedly also.

For various reasons, there has been an increasing trend, in the non-domesticated companion animal trade in amphibians, reptiles, birds and mammals for animals to be captive-bred rather than wild caught. Most marine fish are still wild caught.

The growth in interest in keeping non-domesticated companion animals raises a number of important questions. Is enough known about the husbandry of these animals? Are standards of their welfare satisfactory? Is welfare adequately safeguarded? Does the collection of animals from the wild for the pet industry pose a threat to the viability of wild populations? Does their importation pose risks to indigenous wildlife through introduction of infectious disease or through other adverse impacts if they escape and become established in the wild?

Farmed wild animals and those kept in zoos or for research are protected by specific pieces of legislation (eg Welfare of Farmed Animals (England) Regulations, 2000; Zoo Licensing Act, 1981; Animals (Scientific Procedures) Act, 1986) all of which have mechanisms to set standards for welfare and to update these in the light of growing knowledge of animals' needs. For example, under the Zoo Licensing Act, 1981, standards aimed at protecting welfare are set out in the Secretary of States Standards of Modern Zoo Practice (2000). At present in the UK, there is, however, no corresponding modern legislation for the welfare of companion animals.

Better protection of welfare is likely to be achieved through a combination of improved legislation, education, and the introduction of welfare assurance schemes. Research is needed into the epidemiology and prevalence of husbandry-related diseases and there may be a need to review the risks of possible introduction of exotic infections to indigenous fauna and the adequacy of existing biosecurity arrangements to prevent this.

## Cow's milk components with anti-cancer potential

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**Introduction** Environmental effects are estimated to cause three-fourths of cancer deaths, of which one-third could be reduced by diet modification. Though dairy products are promoted for their nutritional value, and also condemned for alleged health risks, attention is turning to dairy products as sources of functional foods for cancer prevention.

**Milk proteins** Many biological activities of milk components function in a non-specific sense, e.g., whey (and casein) proteins are digested to release encrypted peptides; these have diverse biological properties including opioid activity that potentially modulate various regulatory processes in the body. Dietary whey proteins significantly increase the concentration of glutathione in the liver. Glutathione appears to be an important cancer protective agent, and as such, whey functions as a prophylactic agent, rather than as a therapeutic. Milk  $\alpha$ -lactalbumin is converted to a protein that induces apoptosis in tumor cells. Various peptides in milk are growth factors, e.g., insulin-like growth factor, transforming growth factor  $-\beta$ , and platelet-derived growth factor. Growth factors have diverse effects on cells, e.g., mitogenic, inhibitory to cell growth, regulatory for differentiation and apoptosis. These peptides are stable to pasteurization and stomach acidity; further, they may be absorbed in certain circumstances.

Lactoferrin has iron-binding characteristics; iron, as a pro-oxidant, can generate reactive oxygen species leading to DNA damage. Iron supplementation enhances, and iron chelators inhibit, cell proliferation in several human cell lines. Vitamin-binding proteins in whey enhance absorption of vitamins and include binding proteins for folate, vitamin B<sub>12</sub>, riboflavin, retinal and vitamin D. Folate deficiency is associated with cancers of epithelial tissues; it is essential for proper metabolism and transfer of methyl groups, as is also vitamin B<sub>12</sub>. Methylation of DNA is a key point for regulation of its replication. Riboflavin is a cofactor for enzymes that detoxify carcinogenic azo compounds. Of particular interest is  $\beta$ -lactoglobulin, a whey protein that binds retinal with greater affinity than does retinal-binding protein.  $\beta$ -lactoglobulin also has been shown to bind and increase absorption of vitamin D.

**Calcium** There have been far more human studies, both epidemiological and intervention, on calcium than for all other milk components combined. Many of these have shown a positive response. Milk and dairy products supply the majority of dietary calcium. High calcium diets are effective when initial tumor proliferation rates are high, but not when normal, and may be more effective in high fat diets. Calcium also may prevent recurrence of adenomas that have been surgically removed.

**Probiotics** Saccharides, oligosaccharides, glycopeptides, and glycoproteins of milk promote growth of bacteria that are especially beneficial for gut health and perhaps anticarcinogenesis. These include *Bifidobacterium* and various lactic acid bacteria. Butyrate, a product of their fermentative activity, has antiproliferative, apoptotic and differentiating effects on colon cancer cells, linked to the degree of histone hyperacetylation in the cell nucleus.

**Lipids** Among the milk lipids, rumenic acid (*cis*-9, *trans*-11 CLA) has been documented to be a powerful anti-carcinogen in experimental animals. Dairy products are the single most important source of this fatty acid and its concentration in milk fat is relatively simple to increase. Sphingolipids are complex lipids found mainly in membranes of cells. Dairy products provide about ¼ of the total dietary intake. Hydrolysis products of sphingolipids decrease the number of aberrant crypt foci (precursors of colon cancer) and inhibit conversion of adenomas to adenocarcinomas. Additionally, sphingosine and ceramide affect cell growth, differentiation and apoptosis of cells. Milk fat contains butyrate at about 4-5% by weight. Butyrate has been shown to have antiproliferative, apoptotic and differentiating effects on colon cancer cells, linked to the degree of histone hyperacetylation. Milk fat-derived butyrate is rapidly and efficiently absorbed, followed by equally rapid oxidation in the liver. Nevertheless, butyrate is produced in the colon by microbial action induced by prebiotics and is believed to be protective with respect to colon carcinogenesis.

**Conclusions** Though effects of some milk components as anti-cancer agents are impressive, it is probable that most components act in concert with multiple effects to increase the body's ability to counteract insults of environmental cancer. Most data come from cell culture, laboratory animals or epidemiological studies. Few prospective studies or intervention trials exist and not all data are consistent as to the value of milk components as anti-cancer agents.

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## Dietary manipulation of conjugated linoleic acid in ruminant products

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**Introduction** Conjugated linoleic acid (CLA) is a collective term used to represent positional and geometric isomers of linoleic acid with conjugated double bonds. CLAs have been reported to have a wide range of beneficial effects, including: anticarcinogenic, antiatherogenic, antidiabetic and immune stimulatory. They have been shown to alter nutrient partitioning and lipid metabolism, and reduce body fat in a number of different animal species.

**Synthesis of CLA in the ruminant** Milk and body fat from ruminants contain more than a dozen isomers of CLA and their presence is related to the biohydrogenation of dietary polyunsaturated fatty acids (PUFA) by rumen bacteria. *Cis*-9, *trans*-11 CLA comprises 75 to 90% of total CLA in ruminant fat, and it is an intermediate in the biohydrogenation of linoleic acid in the rumen (Bauman *et al.*, 2001). However, the major source of *cis*-9, *trans*-11 CLA is endogenous synthesis, with *trans*-11 18:1 produced in the rumen as the substrate and the enzyme  $\Delta^9$ -desaturase catalyzing the addition of the *cis*-9 double bond (Griinari *et al.*, 2000; Corl *et al.*, 2001). The *cis*-9, *trans*-11 CLA isomer is anticarcinogenic and has been shown to be effective in a rat breast cancer model when supplied in a natural form (esterified in triglyceride) as a natural food component (butter) (Ip *et al.* 1999). The second most abundant isomer, at 3 to 16% of total CLA, is *trans*-7, *cis*-9, which originates almost exclusively from endogenous synthesis via  $\Delta^9$ -desaturase adding a *cis*-9 double bond to *trans*-7 18:1 derived from rumen biohydrogenation (Corl *et al.*, 2002; Piperova *et al.*, 2002). Other isomers found in ruminant fat make up a very small portion of total CLA and are derived from rumen output. In ruminants, the major tissue sites for  $\Delta^9$ -desaturase and endogenous synthesis of CLA are adipose tissue during growth and the mammary gland during lactation; hepatic levels of  $\Delta^9$ -desaturase are negligible. Several pairs of fatty acids in milk fat represent product/substrate for this enzyme and these have been used to calculate a desaturase index as a proxy for  $\Delta^9$ -desaturase. These include desaturase indexes based on ratios of *cis*-9 14:1/14:0 and *cis*-9, *trans*-11 CLA/ *trans*-11 18:1. Under certain conditions an alteration in the rumen environment occurs to cause a shift in rumen biohydrogenation resulting in a modification of the profile of biohydrogenation intermediates produced in the rumen. One example is *trans*-10, *cis*-12 CLA, a potent inhibitor of fat synthesis in dairy cows, and rumen production of this CLA isomer coincides with dietary induced milk fat depression in dairy cows (Bauman *et al.*, 2001).

**Dietary modification** The majority of studies investigating dietary and physiological factors effecting CLA synthesis in ruminants have been carried out in the dairy cow; studies in growing ruminants are limited. The CLA content of ruminant fat can be increased several-fold by dietary means. Dietary factors that affect this have been grouped into four categories related to the potential mechanisms through which they act (Bauman *et al.*, 2001). The first category includes dietary factors that provide PUFA substrates for rumen production of CLA and *trans*-11 18:1. This typically corresponds to increasing the dietary supply of plant and/or fish oils. The second group consists of dietary factors that affect rumen bacteria involved in biohydrogenation, either directly or via changes in rumen environment. Dietary factors that alter milk fat CLA by modifying rumen biohydrogenation generally affect both Group A and Group B bacteria. For example, modifying the forage:concentrate ratio of the diet typically alters the biohydrogenation of PUFA as previously indicated. The third category includes dietary factors that involve a combination of lipid substrates and modification of rumen biohydrogenation. For example, several investigations have demonstrated that feeding fresh grass to dairy cows doubles the CLA content of milk fat (Lock & Garnsworthy, 2003) and this cannot be fully explained in terms of PUFA supply to the rumen. Other factors or components of grass must promote the production CLA in the dairy cow. The fourth category is dietary supplements of CLA or *trans*-11 18:1 fatty acids. These must be protected from rumen biohydrogenation, typically with calcium soaps or formaldehyde. In the future, the direct addition of CLA to ruminant diets may focus on supplying the *trans*-10, *cis*-12 CLA isomer in order to decrease milk fat synthesis in the dairy cow or decrease fat accumulation in the growing animal.

**Physiological factors** The effects of milk yield and milk fat production on the CLA content of cows milk are negligible. Within herds, animals similarly managed have been shown to have vastly different milk CLA content. Investigations involving diets ranging from maize silage-based total mixed rations to pasture have all reported the milk fat content of CLA varies about 3-fold among individual cows (Lock & Garnsworthy 2002; 2003). The differences among individuals are maintained over the lactation cycle and across dietary shifts designed to produce differences in the CLA content of milk fat (Peterson *et al.*, 2002). The variation among individuals is primarily related to rumen output of *trans*-11 18:1 (and to a lesser extent CLA) and the amount and activity of  $\Delta^9$ -desaturase enzyme. A similar 3-fold variation among individual animals is also observed for desaturase index indicating that individual animals have markedly different levels of  $\Delta^9$ -desaturase despite being managed similarly (Lock & Garnsworthy 2003). Diet composition can affect tissue activity of  $\Delta^9$ -desaturase, and studies have established that gene expression of this enzyme is specifically regulated by insulin and PUFAs (Bauman *et al.*, 2003). Given that individual animals managed in the same way can vary greatly in  $\Delta^9$ -desaturase activity, as demonstrated by differences in desaturase index, a proportion of this variation must have a genetic component.

**Conclusions** The CLA content of ruminant fat is dependent on rumen production of *trans*-11 18:1 and to a lesser extent CLA, and tissue activity of  $\Delta^9$ -desaturase. Future strategies for increasing the CLA content of ruminant products will thus involve three main areas; establishing dietary and nutritional conditions that maximize rumen outflow of *trans*-11 18:1 and *cis*-9, *trans*-11 CLA, optimizing the amount and activity of  $\Delta^9$ -desaturase in mammary and adipose tissue, and identifying the genetic basis for the large differences among individuals in CLA-related variables.

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## Manipulating the diet to reduce environmental pollution from pigs

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**Introduction** In the Netherlands ammonia emission should be reduced considerably to prevent detrimental effects on the environment. Ammonia is mainly formed by enzymatic conversion of urea and other N-compounds in urine. In traditional fattening pig houses approximately 13% of the N taken in by the feed is emitted as ammonia. Different nutritional factors are influencing ammonia emission. Of these the protein content, the acid-base balance and the carbohydrate composition are the most important. In different experiments the sole effects of these factors on ammonia emission have been investigated. The objective of this study was to determine whether the effects of these factors are additive.

**Material and methods** The study consisted of two types of experiments: In-vitro and in-vivo. In the in-vitro experiment different diets were investigated based on combinations of 3 protein levels (142, 161, 180 g/kg), 3 levels of acidifying salt (2.2, 6.9, 11.6 g/kg  $\text{SO}_4^{2-}$ ) and 3 levels of digestible fermentable carbohydrates (62, 83, 104 g/kg). These diets were given to pigs on balance cages. Urine and faeces were collected and mixed and ammonia emission was determined in an in-vitro set up. On basis of the first experiment 3 diets were selected and the in-vitro results of these diets were validated in a real pig house.

*In-vitro experiment* For this experiment 39 castrates were used. The experiment was run in 3 periods with 13 pigs. A Box-Behnkin design was used to efficiently run the experiment. New pigs were used for every period. Daily feed and water was restricted. The water to energy ratio was fixed. Animals were housed in individual metabolic cages. Each balance period consisted of an adaptation period of 3 weeks and a main period of 5 days. In the main period urine and faeces were collected quantitatively. They were mixed and added to a vessel of 40 l during 5 days at a temperature of 20°C. At the end of the collection period a representative sample of 2 kg manure was taken and used for the in vitro measurement of ammonia emission. The manure samples were added to vessels of 6.5 l in a room at 20°C. A lid closed the vessels. Ventilation rate of the vessels was 4.0 l/min. Air was withdrawn from the vessels and ammonia was stripped from the air by an acid solution. Ammonia concentrations in the acid solutions were cumulatively determined during 7 days. Results were analysed with a linear model in which linear and interaction effects were calculated.

*In-vivo experiment* In-vivo the following diets were tested: diet 1 with a high level of protein and low levels of  $\text{CaSO}_4$  and digestible fermentable carbohydrates; diet 2 with medium levels of these components and diet 3 with a low level of protein and high levels of  $\text{CaSO}_4$  and digestible fermentable carbohydrates. Each of the three experimental groups consisted of 72 pigs with initial weight of 30 kg. The experiment was done in 2 periods. In between the periods the diets were exchanged between groups. Each period consisted of an adaptation period of 2 weeks and an experimental period of 3 weeks. Animals were restrictedly fed with wet feed. Ammonia concentration and ventilation rate of the outgoing air were measured.

**Results** The in vitro study of ammonia emission showed that ammonia emission was linearly related to the levels of protein,  $\text{CaSO}_4$  and fermentable carbohydrates in the diet. The effects could be described with the following model:

$$AE = -5.747 (0.656) + 0.056 (0.0038) * P - 0.050 (0.0091) * AS - 0.010 (0.0013) * DFC$$

Where: AE=ammonia emission (g); P=protein (g/kg); AS=acidifying salt ( $\text{SO}_4^{2-}$ , g/kg); DFC=digestible fermentable carbohydrates (g/kg)

Reduction of the protein content from 180 to 142 g/kg resulted in an ammonia emission reduction of 63%. When increasing the sulphate concentration from 2.2 to 11.6 g/kg, in the form of  $\text{CaSO}_4$ , ammonia emission was lowered by 25%. When the digestible fermentable carbohydrate concentration in the diet was increased from 83 to 104 g/kg ammonia emission was lowered by 6%.

In the in-vivo experiment the results of the in-vitro experiment were confirmed. Diet 2 with medium levels of protein,  $\text{CaSO}_4$  and DFC reduced ammonia emission by 44% when compared to diet 1 with a high level of protein and low levels of  $\text{CaSO}_4$  and DFC. Diet 3 with a low level of protein and high levels of  $\text{CaSO}_4$  and DFC resulted in a reduction of ammonia emission of 69% when compared to diet 1.

**Conclusion** From these experiments it is concluded that combining different feeding measures (lowering protein content and increasing the levels of acidifying  $\text{CaSO}_4$  and digestible fermentable carbohydrates) can reduce ammonia emission from pig houses considerably. The different feeding measures affect ammonia emission independently, so effects of the sole factors are additive.

## **PCV2/PMWS field studies, experimental infections and immunological parameters**

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**Introduction** The Virology Department, VSD, DARDNI, in collaboration with colleagues in Europe and N America has had an active research programme on porcine circovirus-related diseases, including PMWS, for the last 5 years. This presentation will highlight some of our on-going research in this area.

**Epidemiology** Antibody to a PCV2 virus is widespread in pigs throughout the world. Retrospective analysis of sera from 1974 has also shown the presence of antibody to a PCV2 virus in a high percentage of the sera tested [Walker et al., 2000]. Seroconversion to PCV2 was demonstrated in pigs on commercial farms between 3 and 4 weeks post weaning (Cotrell et al., 1999). PCV2 nucleic acid is detected in faeces, urine and blood samples of young pigs on PMWS -affected and PMWS-unaffected farms. Transmission of PCV2 infection from experimentally-infected pigs to seronegative pigs in separate but neighbouring pens has been demonstrated (Charreyre et al., 2000). Evidence of vertical transmission of PCV2 has recently emerged in association of PCV2 with abortions in Canada (West et al., 1999) and Denmark (Ladekjaer-Mikkelsen et al., 2001). PCV2 nucleic acid has been demonstrated in semen from boars experimentally infected with PCV2 and field boars. The consequences of sub-clinical vertical transmission have not been investigated.

**PCV2-associated diseases (field observations)** PCV2 has been associated with a number of disease syndromes in pigs (Allan and Ellis 2000). PMWS was first identified in 1991 in high health SPF herds in Western Canada (Clark, 1997). PCV nucleic acid and antigen was demonstrated in abundance in the lesions of affected pigs and subsequent isolation and characterization of a PCV2 virus from diseased pigs was reported (Ellis et al., 1998) (Fig 3). PMWS is now a global pig disease. PCV2 antigen has been demonstrated in lungs from pigs in Canada, N Ireland and Scotland with proliferating and necrotising pneumonia (PNP), in pigs with sow abortion and mortality syndrome (SAMS) and in lungs from pigs previously misdiagnosed as having post-weaning PRRS (Allan and Ellis 2000). PCV2 is now recognised as a viable causal agent of reproductive disorders in pigs and has also been associated with porcine dermatitis and nephropathy syndrome (PDNS). Recent reports from the USA have identified PCV2 as an important agent in porcine respiratory disease complex (PRDC).

**PCV2-associated disease (experimental observations)** Gross/histological lesions consistent with PMWS were first reported in PCV2 and porcine parvovirus (PPV)-experimentally infected gnotobiotic pigs in 1999 (Ellis et al., 1999). In a study in CD pigs, PMWS was reported in 1 of 4 piglets inoculated with PCV2 alone (Allan et al., 1999) and severe disease was seen in all pigs inoculated with PCV2 and PPV. Pigs inoculated with PPV alone were clinically normal. In a similar experiment in gnotobiotic pigs that used the same inoculi, clinical diseases was seen in 100% of PCV2/PPV dually infected pigs and no clinical diseases in pigs inoculated with PCV1, PPV, PCV2 alone, or in pigs inoculated with combinations of these 3 viruses (Krakowka et al., 2000). Recent studies in this gnotobiotic model have shown that inoculation of pigs with PCV2 alone + non-specific immune stimulation results in PMWS in 100% of the inoculates. The authors concluded that PCV2 was the only causal infectious agent necessary for the development of PMWS (Krakowka et al., 2001). PMWS has now been experimentally reproduced in CD piglets using a PCV2 isolate from clinically normal pigs from Sweden (Allan et al., 2002).

**Immunopathogenesis** In-vivo studies on tissues from PMWS-affected pigs have shown that macrophages and/or dendritic cells contain large amounts of PCV2 antigen. Recent in-vitro studies have confirmed that PCV2 does not replicate, nor is it degraded, in these cell types. Stimulation of the immune system of young pigs, shown experimentally to be a "trigger" for PMWS in current husbandry practises can be multifactorial. PMWS-affected pigs (experimental infection) develop a clear leukopenia. CD 21+ve B and CD3+ve T lymphocytes are affected with the loss of CD3+CD4+CD8+ve memory/activated Th lymphocytes particularly notable Granulocytes and monocytes are less affected. C reactive protein (CRP) levels increased in gnotobiotic pigs in the early stages of PCV2 infection (0-16dpi) and high in conventional pigs affected by PMWS. Cytokine levels (TNF, IL-1, IL-4, IL-6, IL-8) in gnotobiotic pigs are elevated in the presence of PCV2 during the early stages of infection (0-16 dpi). In conventional pigs affected with PMWS IFN alpha levels were decreased whilst levels were maintained in PCV2 infected, but healthy animals

**PCV2: The future** Our understanding of PCV2-associated diseases has advanced in the last few years, however a number of important questions still remain to be answered. We have not identified the primary site of replication of PCV2 in pigs, nor do we know the mechanism/s of potentiation of PCV2 replication following immunostimulation. In addition, we still do not know if sub-clinical vertical transmission occurs, and if it does, how that affects the eventual clinical condition of the pig. We have no information on serological differences between PCV2 isolates (if they exist), age resistance to disease associated with PCV2 infections or the role of pig genetics in the susceptibility of pigs to PCV2-associated disorders. More research is needed in these areas if we are to fully understand PCV2 interactions with the pig and, in particular the pig immune system.

## **Clinical presentation, epidemiological findings, diagnosis, immunity, prevention and control of postweaning multisystemic wasting syndrome (PMWS)**

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**Introduction.** Postweaning multisystemic wasting syndrome (PMWS) was initially described in 1991 in Saskatchewan (Canada) and has now been described in all continents rearing pigs but Oceania. Porcine circovirus type 2 (PCV2) is the aetiology of this disease, which has also been called porcine circovirus in some countries. Although the full spectrum of clinical signs and lesions observed in natural cases of PMWS is very difficult to reproduce under experimental infections using PCV2 alone, little doubt exists on the causal relationship between the virus and the wasting syndrome. Furthermore, the clinical and pathological scope of PCV2 infection has been expanded since 1991, and it has been implicated in other conditions: reproductive disorders, porcine dermatitis and nephropathy syndrome (PDNS), the so-called porcine respiratory disease complex (PRDC), proliferative and necrotising pneumonia (PNP), and congenital tremors. The role of PCV2 in these conditions has not been fully clarified and, in some of these cases, it remains as a controversial issue. The objective of this presentation is to review some practical aspects of PMWS.

**Clinical presentation.** PMWS most commonly affects pigs of 2 to 3,5 months of age, although the disease has been described in 1 to 6 month-old pigs. Morbidity and lethality are variable depending on the farms and on the batches of animals, being the usual rates are 4–30 % and 70–80 %, respectively. Major clinical signs of the disease include wasting, unthriftiness, pallor of the skin, respiratory distress and, sometimes, diarrhoea and icterus. An individual expression of the disease seems to be a key point in this syndrome; in a given pen, only some individual pigs exhibit clinical signs. These pigs tend to die or to develop marked wasting within a few days. The different treatments, using mainly antibiotics, fail to counteract the disease. In a French on-farm study, it was observed that most of the pigs developing PMWS corresponded to a few litters, suggesting a possible litter effect. Another French report showed that castrated male pigs were more susceptible to PMWS than females; moreover, in the same French study, it was observed that pigs with lower birth and weaning weights tended to develop PMWS with higher frequencies, as well as the lighter pigs at the beginning of the fattening period. Concurrent infections are very frequent in severely PMWS affected pigs.

**Epidemiological findings.** PMWS has been described in almost all types of farms, including farrow-to-finish and multi-site operations, and sizes from 30- to 10,000-sow herds. From a serological point of view, PCV2 infection is spread worldwide. Furthermore, data from different European countries showed almost 100% herd sero-prevalence, indicating that PCV2 infection occurs in both PMWS affected and non-affected farms. In herds where PMWS is present, mortality is clearly associated to PCV2 sero-conversion. PCV2 detection in serum of individual pigs may vary from 5 to 21 weeks of duration (with continuous or intermittent detection) suggesting that a percentage of pigs may develop a fairly persistent viremia. However, no direct association has been established between viremia, length of this viremia and development of PMWS

**Diagnosis.** A pig or a group of pigs are considered to suffer from PMWS if they accomplish the following criteria: 1) presence of a clinical picture compatible with PMWS, 2) presence of characteristic histopathological lesions in lymphoid tissues (lymphocyte depletion together with granulomatous inflammation; a percentage of cases have multinucleate giant cells and PCV2 intracytoplasmic inclusion bodies) and 3) detection of PCV2 within the lesions in tissues of affected pigs. In all PMWS cases, PCV2 is present in a variable amount at least in one tissue, always closely associated to the characteristic microscopic lesions.

**Immunity.** Most of the knowledge associated to the immune system in PMWS affected pigs suggests that severely affected animals are immunosuppressed. In fact, these data are not in contradiction with the suspicion that, an apparently contradictory phenomenon like immunostimulation, may be needed in PMWS. It seems that immunostimulation could be a triggering factor for the development of the syndrome in certain circumstances, while immunosuppression seems to be the outcome of severely affected pigs.

**Prevention and control.** Since no vaccine products are readily available against PCV2, generic zootechnical changes have been proposed to reduce the so-called “infection pressure” regarding PCV2 and any other pathogen in order to control PMWS. The changes mainly concern an improvement of hygiene and a reduction of stress at the different stages. These measures include the reduction in mixing pigs, adequate pig flow (strict all in - all out procedures) and pig density, special care when castration, and improvement of air quality and comfort during the postweaning and growing periods. Other measures include cross-fostering, if necessary, only in the first 24 hours after birth, to avoid using big-size pens, to use openwork partitions between pens, to segregate sick animals as soon as possible to hospital facilities, to have sick pens located out from the nursery or growing unit, and to medicate with antibiotics (injectable) only sick animals, at least during 3 consecutive days. Other suggested strategies to minimise the impact of PMWS may include the use of immunomodulators, feed-back with viscera and blood of affected animals and serum-therapy. The latter has been considered successful in a percentage of PMWS cases.

## **Management system to control and minimise effect caused by post-weaning multi systemic wasting syndrome and porcine dermatitis and nephropathy syndrome**

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**Introduction** The increasing prevalence of PMWS /PDNS within global pig production since 1995 has steadily increased, UK first affected in 1999. Impact on post weaning mortality is variable with rates exceeding 30% commonly reported, no medication or treatments have proved effective or successful, vaccination is still unavailable and the industry is still striving to find the scientific answers and solutions behind this economically damaging disease. Porcine Circovirus 2 (PCV2) is widely acknowledged as the probable causal agent but still not confirmed, if so has it changed or is it a new PCV? With enhanced virulence, a definitive identification and test is urgently required. The objective is to demonstrate how successful specialised management techniques alongside a batch system is capable of reducing post-weaning mortality to pre PMWS levels and to further improve overall herd health, productivity and economic performance.

**Materials and methods** Unit of 500 productive sows having clinical signs of PMWS / PDNS for the period Jan – Dec 2000, average post weaning mortality of 18.6%. Substantial in-feed medications and soluble treatments were carried out during the 12 months at a cost of £28,000. The failure of medication to impact on mortality caused major concern, a review of all management practices was undertaken with a decision to cease all medication treatments specific to PMWS / PDNS. Changing production system from weekly continuous flow will allow greater segregation of ages and groups to give greater impact of new protocols. Three Weekly Batch Production was planned with key objectives, to create all in all out at every stage for all progeny, reduce stress for all pigs, improve environmental conditions, implement disciplined hygiene routines and improved management practices. Using the basic principles of F. Madec (1998) 20 points plan, Rattlerow have adapted these recommendations to suit units in the UK. Partial de-population of the entire unit was necessary by ceasing serving for four weeks and culling all sows at weaning. The upgrading of health status was not considered an option due to the close proximity of other units in the locality. Change of breed from pure Large White females to Landrace x Duroc females and Large White males (100% use of AI) was taken as a commercial decision with no relevance placed upon any relationship between genotype and disease resistance. Health status of new herd gilts is Enzootic pneumonia (EP)+ve, Porcine reproductive and respiratory syndrome (PRRS)+ve and PMWS / PDNS +ve. This gives an accurate health match with existing herd, under new management guidelines vaccination programs are put into place to minimise risk from secondary infection. EP vaccination is carried out routinely on all progeny with PRRS vaccination carried out as necessary (subject to changing status) Application of Regumate is a key factor in synchronising oestrus in large groups of gilts to establish initial first full cycle of seven batches and replacement gilts thereafter. Oil based product containing the active principle altrenogest a progesterone substance, feed daily for 18 consecutive days (5ml placed on feed) following withdrawal gilts come on heat 5-7 days later.

**Results** As the service program progressed it became apparent the expected farrowing rate would exceed expectations due to the success of the Regumate program. Results from the initial 516 gilts that received Regumate show success on 500 (representing 96.89%) the overall farrowing rate was a creditable 91.66%. The four week break in serving meant at least one building was empty between the old and the new herd progeny, strict discipline procedures for the movement of staff and equipment between both feeding herds within the same site minimises cross contamination. Trials show significant improvements in growth rate from ADG of 378 gms @ 35.89kg from birth to 91 days, to an ADG of 488 gms reaching 45.95kg at the same age (represents increase ADG of 110 gms) This level of improvement has remained constant, the unit has completed its fourth parity with NO clinical signs of PMWS /PDNS, post weaning mortality has returned to pre disease levels. Financial results have dramatically improved from a breakeven of £67.42 during initial 12 months post PMWS/ PDNS down to £53.23 for 12 months to end of September 02 (reduction of £14.19 per pig sold) the greatest contributing factor is due largely to increased pig sales versus decreased mortality (21.91 against 18.32 = 3.59 extra pigs sold per sow year)

**Conclusions** PMWS /PDNS has highlighted the urgent need for all pig producers to look at their existing production methods, systems which are outdated and do not have the principles of what is considered best for the pig will continually struggle against disease. Those that apply three weekly batch production will not only manage to control this disease but will succeed in minimising the effects of others, from an economic perspective pig producers who allow their pigs to express full genetic potential will be rewarded with reduced cost of production. Three weekly batch production, has now become the 1<sup>st</sup> choice production system for Rattlerow Farms.

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## Genetic improvement programs for aquaculture species

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**Need for genetic improvement programs** The high yields obtained in agriculture rely heavily on the use of domesticated and genetically improved breeds and varieties. Until quite recently this has not been the case for most farmed aquaculture species that, in the genetic sense, are still much closer to the wild state than are the major terrestrial animals and food crops. Less than 10 % of the total world aquaculture production is based on improved strains. Due to a growing human population and a decline in production from capture fisheries, there is therefore a great disparity between the need for increased aquaculture production and the genetic quality of the strains available to meet that need. Moreover, full benefits of investments in management improvements (feed and feeding practices, control of diseases, etc.) can only be obtained through the use of genetically improved animals.

**Potential for genetic improvement** The prospects of genetic improvement of economically important traits are well documented in several fish species (Gjedrem, 1997). For instance, for growth rate, genetic gains of about 10% of the mean per generation are frequently reported, implying that growth rate can be doubled over a period of seven to eight generations. In general, estimates of heritability in fish species are within the range of those observed for terrestrial species while the magnitude of non-additive genetic effects has been shown to be much more important than previously assumed. This potential for improvement should be exploited through effective and sustainable programs to develop strains for aquaculture with improved performance, resource efficiency and product quality.

**Special characteristics of fish** The very high fecundity and the possibility of collecting eggs and semen separately in many species facilitates a wide range of mating designs, and allows very high short-term genetic gains through intense and accurate selection. On the other hand, the use of few breeding animals can lead to a rapid accumulation of inbreeding and therefore implementation of measures for restricting inbreeding is essential. In order to control inbreeding and to use information on relatives in selection decisions (and therefore for increasing accuracy) genetic relatedness needs to be monitored. However, a specific problem in fish breeding programs is the difficulty of uniquely identifying individuals at hatching due to their small size. Keeping families separated until the fish are large enough to be individually tagged overcome this but to a high cost since it limits the number of families available for selection and can induce environmental effects common to the members of the same family. Although the technology is still expensive, DNA-based markers (e.g. microsatellites) can be used to solve this problem by allowing discriminating fish in mixed family groups.

**Opportunities and challenges** The first large scale selective breeding program for farmed fish was set up for Atlantic salmon in the nineteen seventies (Gjøen and Bentsen, 1997). Its design was based on basic knowledge in quantitative genetics, experiences from livestock programs and available technologies. Full-sib families were reared separately until family identification was obtained by cold-branding and fin-clipping. With the exception of some improvements (e.g. individual PIT tagging at a smaller size), rather few changes have been taken place since then. Other family based programs for salmonids and other species, developed mainly in the late nineteen eighties and nineties, have followed, to a large extent, the same design. Presently, 27 improvement programs that use sib information in the selection decisions are in operation in the world today involving nine different species.

The opportunities and challenges in designing sustainable fish breeding programs have been discussed in general terms (Gjerde *et al.*, 2002), but studies on optimum designs are few and limited to mass selection programs. The design of current programs is thus only partially based on well-defined scientific grounds and research is needed to adapt and develop new theory and tools to account for the special reproductive characteristics of fish species and for the impossibility of individual identification at hatching. In particular, research is required for determining how best 1) to establish base populations with broad genetic variability; 2) to define mating and selection decisions for maximising gain while restricting inbreeding; 3) to create sufficient connectedness between generations, sub-populations and cohorts; 4) to exploit non-additive genetic variance; 5) to use DNA markers; and 6) to disseminate the improved genetic material to the industry.

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## Aquaculture nutrition – a brief and topical review

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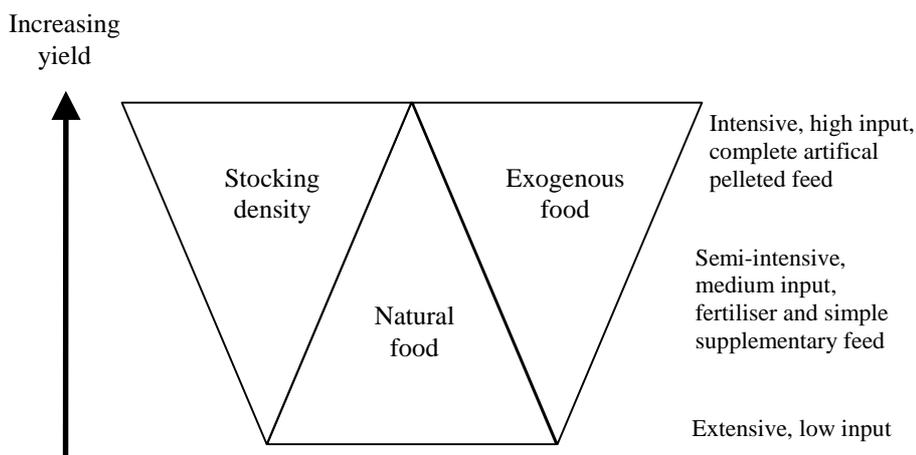
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**Introduction** Aquaculture is a rapidly expanding and extremely diverse form of animal production. Aquaculture takes place in systems that cover an enormous range of levels of intensification from extensively managed low input static ponds to super-intensive high exchange rate silos with liquid oxygen injection. Appropriate nutritional strategies are required for each level of intensification.

Aquaculture nutritionists also face challenges resulting from the diversity of aquatic animals in culture. Although popular perception is of fish farming, the industry also produces crustacea, amphibia and occasionally even reptiles. Within each of these groups there is a wide range of preferred natural feeding types from planktivores (both carnivorous and herbivorous), through herbivores grazing on aquatic macrophytes, then widely distributed and successful omnivores to top carnivores. In contrast to terrestrial farmed animals, most of the high value aquatic species farmed for luxury/export markets are carnivores. Each type of natural feeding specialisation has led to the evolution of appropriate, and highly varied, physiology that also impacts on how these animals may be fed in culture.

Aquaculture nutritionists face other challenges imposed by the extreme variability in the physical and chemical environment to which farmed aquatic animals are exposed. As ectotherms water temperature naturally has a very large effect on metabolism of aquatic animals but variables such as dissolved oxygen level, salinity, pH, hardness etc. all have effects that can directly or indirectly affect nutrition.

**Level of intensification** Most of the world's aquaculture is semi-intensive and is based in pond systems with little or no water exchange where a significant proportion (sometimes all) of the nutrient requirements of the farmed animals are supplied from the pond ecosystem. When the stocking density/yield increases, a point (the critical standing crop) will be reached at which natural food alone (even if boosted through fertilisation of the pond with inorganic or organic fertilisers) will not meet nutrient requirements. Supplementary feed (cheap energy rich materials, generally cereals and cereal by-products) must then be supplied. If the stocking density/yield continues to increase the exogenous food must meet an increasing proportion of the nutrient requirements until a point is reached at which a complete feed is required.



**Figure 1** Relationship between yield, stocking density and relative contributions to production from natural & exogenous food

**Issues in fish nutrition** Fish are very different from farmed terrestrial animals and this paper will address a few of the more interesting issues; fish have proportionately small skeletons (neutral buoyancy) and high 'dress-out' weights; fish are ectotherms leading to a high dietary protein:energy ratio (efficient use of dietary energy); fish obtain nutrients (and excrete) by passive or active transmission across the gills (and sometimes the body surface); living in water imposes experimental constraints (food intake is difficult to measure, food water stability is important, excretions are difficult to collect, biomass is difficult to measure). Fish, in general, resemble diabetic higher animals and thus have problems with carbohydrate nutrition. An important group of cultivated fish (the carps) are agastric requiring a different approach to nutrition. Given the high concentration of protein required in fish feeds, there is considerable dependency on fishmeal as a protein source and there are issues about substituting this, especially in diets for carnivorous fish. Many fish require long-chain n-3 ( $\omega$ 3) fatty acids as EFA as well as being the main source of these in the human diet leading to dependency on fish oil as a lipid source. Commercial feeds for salmonids now frequently contain  $\geq 35\%$  lipid and fish oil supply (and substitution) is becoming a major issue.

**Conclusions** Even though much is now understood about fish nutrition this area of science is still in its infancy compared, for example, to broiler nutrition. More is known currently about the required nutritional characteristics of fish feed than is known about how to best feed these diets (feeding husbandry is only recently being explored in any depth). There are enormous opportunities and challenges still facing aquaculture nutritionists, especially with respect to supporting an industry that is sustainable and environmentally acceptable in the longer term. If we become complacent there will always be new species to challenge us.

## **Health management in aquaculture**

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The management of disease in fish presents many challenges which are not usually encountered in terrestrial animals. The aquaculture environment poses severe limitations on general observation, examination, disease prevention and treatment. Aquaculture has been described as 'three dimensional farming' with recognition of health problems often reliant on surface observation, although the use of remote control camera systems is increasing. The ability to carry out adequate observation may be restricted by poor access or adverse weather.

Investigation of possible disease problems is frequently initiated by changes in feeding or swimming behaviour followed by appropriate sampling and analysis. As many symptoms are common to a variety of diseases it is usually necessary to carry out extensive laboratory analysis which may include histopathology, bacteriology, virology and water analysis. Some of these techniques are protracted and may result in delay in control and treatment.

In comparison with other intensive animal production systems where the animal's environment can be very carefully controlled, most fish farms are exposed to the natural aquatic environment and many of the infectious and parasitic diseases in farmed fish are caused by organisms which are carried asymptotically in wild fish. Farmed fish are also very vulnerable to changes in the physical and chemical characteristics of the water in which they are farmed as well as incidents such as pollution and algal and jelly fish blooms.

There are very few effective therapeutic agents available for the treatment of fish disease; it is a relatively small market and few companies are willing to invest in the development and registration of products for the aquaculture sector where there is also a need to satisfy very stringent environmental requirements set by the environmental agencies. The very lack of available effective treatments for many fish diseases requires health management to centre on careful management and husbandry, stress-free handling and attention to fish welfare.

Many fish farming companies have in-house diagnosis and health management services. These are often essential due to the remote locations of many farms and lack of locally available veterinary services. Company health personnel usually liaise regularly with their veterinary surgeons (some farming companies also employ vets) to ensure comprehensive fish health cover and the relationship between the farm and their veterinary surgeon is set out in veterinary health plans which are also an essential component of the many fish quality assurance schemes which are playing an increasingly important part in the production of farmed fish.

## Microbial community analysis and its application in gastrointestinal health

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**Bacteria in the gastrointestinal tract.** The intestinal microflora are an integral part of the digestive system of all animals. Bacteria in the gastrointestinal tract derive most of their energy for reproduction and growth from dietary compounds, which are either resistant to attack by digestive fluids or absorbed so slowly that bacteria can successfully compete for them. Since bacterial species differ from each other in relation to their substrate preferences and growth requirements, the chemical composition and structure of the digesta largely determines the species distribution of the microbial community in the gastrointestinal tract. As a consequence, bacterial community structure is very much dependent upon the diet as the ultimate source of substrates for metabolism.

**Microbial community analysis.** The ability to accurately monitor changes in the microflora depend upon the specific methods used. It is important to note that methods available for monitoring the total microbial community of the GI tract are few. All bacteria big enough can be seen under fluorescence microscopy, but only a few of them can be cultured under laboratory conditions. Indeed, most of the bacteria growing in such a complex community are dependent upon growth factors provided by other community residents or the secretions from the host tissues. As a consequence, most of the work and conclusions compiled until now reflect only minor members of the gastrointestinal microflora. Many diseases, the cause of which is not known today, may have a causative agent among the yet unknown, unculturable majority of the gastrointestinal tract microbes.

Our laboratory is using mainly DNA based approaches for analysing microbial communities in the gastrointestinal tract. DNA of the total intestinal bacterial community is first recovered from the sample by using a process designed so that it should not discriminate for any bacterial types; that is, the method depicts faithfully the total rather than a partial bacterial community. Specificity, when desired, comes from the discriminating methods used for DNA analysis. The specificity ranges from the total bacterial community to bacterial strain level, depending on the technique used. Presently we are using techniques based on guanine + cytosine content of the bacterial DNA, hybridisation (DNA arrays), polymerase chain reaction methods with specific primers and sequencing of the 16S ribosomal DNA, in the order of increasing specificity(2, 5, 8). It is worth noting that no single technique can show the picture of the total bacterial community and be species specific at the same time.

**Modulation of bacterial community.** Intestinal health is now one of the most important topics in Europe, where the use of antibiotics on a prophylactic basis has been severely curtailed in an attempt to limit the spread of antibiotic resistant zoonotic organisms. As reliance on growth promoting antibiotics for maintaining animal performance and health diminishes, it is clear that more attention will need to be paid to alternative means for maintaining intestinal health. Specific species can be selected for by certain feed ingredients, which escape digestion by the host, but are readily available for the metabolic machinery of the target microbes. In addition to some structural components of compound feeds, special products belonging to this group include prebiotics, dietary fibre, oligosaccharides etc. We have demonstrated several means to modulate microbial community in the gastrointestinal tract of various animals.

Modulating gastrointestinal microflora straightforward, but it is not presently possible to assess the physiological significance of all the bacterial shifts detected by the methods described above. However, the availability of relatively fast methods for monitoring of total bacterial communities is a prerequisite for informative epidemiological surveys in the future. The type of data shown in this paper could be correlated with other parameters reflecting animal performance and health (e.g. productivity, immune status, bacterial metabolites) and together would greatly improve our understanding on gastrointestinal interactions and the importance of the microbial community structure.

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## Gut morphology and nutritional management

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**Introduction** In the natural situation, the piglet's transition from a wholly milk to a wholly non-milk diet (weaning) is gradual and paralleled by progressive, appropriate modification of gut structure. The sow's milk provides nutritional, trophic and immune support throughout this transition thereby mediating intestinal exposure to novel nutrients and pathogenic agents whilst the gut structures necessary to process them appropriately are maintained and developed. Commercial weaning deprives the piglet of lactogenic support and simultaneously necessitates the marked acceleration of gut development if piglet health and growth are to be maintained. Intestinal mass, growth (Slade and Miller 2000) and fractional protein synthesis rate (Ks) increase significantly following weaning (Le Dividich and Seve 2000). Correspondingly, the nutrient cost of post-weaning intestinal development is high. However, weaning is invariably associated with a dramatic reduction in feed intake and, in turn, with a decline in gut efficiency characterised by a reduction in villus height and an increase in crypt depth. Perversely, the gut morphology of the weaner thus appears a limiting factor in its own development. Here we introduce a simple histological method for estimation of individual crypt-villus structure surface area and then, in conjunction with conventional histomorphological measurements, use this to relate piglet performance, nutrition and gut morphology.

**Crypt-villus surface area** The negative effects of post-weaning villus atrophy on intestinal absorptive area are to some extent compensated for by concomitant increases in crypt depth and villus diameter, and in intestinal length and diameter. The crypt villus structure is essentially a tube of height  $X$  and diameter  $Y$ ;  $X$  being the distance from the crypt basin to the villus apex and  $Y$  being the mean width of the villus. In our experiments we have found that villus width measurements taken 100 $\mu$ m above the crypt-villus interface were similar ( $P>0.1$ ) to the mean value of six measurements taken equidistantly between the crypt basin and the villus apex. Thus an estimate of the crypt-villus surface area can be obtained by simple geometric calculation ( $X \times \pi Y$ ).

**Porcine plasma** Although not used in UK weaner rations, inclusion of porcine plasma in the postweaning diet is frequently associated with transient improvements in early performance. These benefits are most pronounced in circumstances where the baseline weaner performance is poor either due to low hygiene (Coffey and Cromwell 1995) or high reliance on soy proteins in the diet (van Dijk et al. 2001). Thus, in situations or periods of increased intestinal stress, plasma is beneficial. However, research carried out at Leeds indicates that, even when compared with a typical UK high milk protein content weaner ration, the gut morphology of pigs fed 7.5% porcine plasma for 7 days was significantly improved. Plasma significantly increased villus height, villus width and, interestingly, crypt depth. Crypt-villus surface area was subsequently greatly enhanced and beneficial effects on feed intake and average daily gain were observed. However, plasma improvements in gut morphology were exclusive to the proximal third of the small intestine. Medial surface area tended to be greater in non-plasma fed pigs but distal response was unaffected by dietary treatment.

**Whey globulin concentrate** The use of milk-derived sources of protein in the diet of newly weaned pigs is common throughout Europe. Milk proteins are high quality, readily digestible and, importantly, non-controversial. Substitution of whole ovine or bovine milk for sow milk at weaning maintains preweaning gut morphology (Pluske, Williams and Aherne 1996a; Pluske, Williams and Aherne 1996b). Supplementing the weaner diet with whey globulin concentrate for four days following weaning positively influences subsequent performance (Miller and Toplis 2000). Further studies carried out with individually housed animals indicate that short-term whey globulin supplementation results in subsequent enhancement of medial crypt-villus surface area and that this is achieved primarily through reduction in villus atrophy. In contrast to the gut response to plasma, whey globulin had no apparent effect on the morphology of the proximal small intestine. Development of the distal gut structure was again unaffected by dietary treatment.

**Nutrient quantity and quality** The detrimental changes in gut morphology initiated following weaning are thought to be influenced by the level of nutrient intake, yet studies in which feed intake level has been related directly to the changes in gut morphology are rare. Our studies indicate that the gut morphology of the proximal and medial regions of the small intestine are significantly affected by protein source in the diet. The lack of a plasma or whey globulin effect on development of distal gut morphology suggests their comprehensive utilisation in the proximal and distal regions of the small intestine. However, feed intake level and morphological development of the distal small intestine are strongly correlated.

**Conclusions** These findings suggest the exciting possibility that we could direct regionalised development of the gut by selection of specific components in the diet. Further work is required to identify these components, to define the mechanisms by which they stimulate trophic responses and to establish the benefits associated with development of particular regions and structures in the gut.

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## Dietary carbohydrates and management of the gut environment of pigs

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**Introduction** Dietary carbohydrates constitute a major fraction of the diets for pigs. The carbohydrate fraction consists of mono-, di- and oligosaccharides and two broad classes of polysaccharides – starch and non-starch polysaccharides (NSP). The carbohydrate fraction has a diverse composition in terms of constituent sugars (pentoses, hexoses, deoxysugars, etc.), glycosidic linkages (alfa or beta), size (degree of polymerisation from one to several thousand), and physical form (soluble in water, insoluble, cation and adsorbing properties). It is now evidential clear that the composition of the carbohydrate fraction influences the digestion and absorption processes of carbohydrates and other nutrients in the various parts of the gastrointestinal tract, it has a profound influence on the secretory response of the gut to feed intake, the volume flow, the mucosal architecture, the composition of the gut flora and the development of the gastrointestinal tract.

**Oligosaccharides** The intestinal mucosa of the pig lacks the enzymes capable of cleaving a number of oligosaccharides that are naturally present in plant materials (i.e. raffinose-oligosaccharides, fructooligosaccharides) or used as feed additives (i.e. neosugar, transgalactooligosaccharides). Collectively, these oligosaccharides are referred to as non-digestible oligosaccharides (NDO). NDO were earlier considered as an antinutritional factor, which could potentially accumulate in the small intestine, cause osmotic diarrhoea, and, because of their rapid fermentation and high gas production, cause discomfort for the animals. Recently, there has been a growing interest in NDO because of their possible prebiotic properties, i.e. stimulation of the growth and (or) activity of one or a limited number of desired bacteria in colon and thus, exclude the pathogens. In man, it has been demonstrated that inulin and oligofructose independent on chain length have significant prebiotic properties by selective stimulating of the growth of bifidobacteria in the colon. The results from studies with pigs, however, are less convincing and it seems as if the concept, because of economically constraints, can be difficult to use for older pigs.

**Starch** is the most abundant carbohydrate in diets for pigs. Starch is a mixture of the linear  $\alpha(1-4)$ - linked amylose and the branched  $\alpha(1-4),(1-6)$ - linked amylopectin. Most of the ingested starch is efficiently broken down by the combination of secreted  $\alpha$ -amylase and enzymes located on the intestinal surface membrane. A variable fraction of starch (resistant starch, RS), however, will be resistant against degradation in the small intestine and may have properties similar to NSP. A high amylose cornstarch diet fed together with *Bifidobacterium longum* has been shown to increase the number of bifidobacteria in the faeces compared to a low amylose cornstarch diet. Studies with catheterised pigs also show that high levels of RS change the composition of short-chain fatty acids produced towards more butyrate.

**NSP** are the main carbohydrate fraction not digested by enzymes in the small intestine of pigs. At this site of the gastrointestinal tract, NSP composition (type of polysaccharides, solubility) will have a major influence on the physicochemical properties of the digesta materials. However, because of the microbial colonisation of the stomach and small intestine, some disappearance (~24 %) of NSP occurs in the upper intestinal tract. Results obtained with cereal diets consistently show a higher degradation of the linear and relatively soluble  $\beta$ -glucan compared to the branched-chain arabinoxylans from wheat, rye and oats. The molecular weight of  $\beta$ -glucan was reduced up to 20-fold in the upper gastrointestinal tract but with relatively low digestibility until the terminal ileum. The amount of dry matter that passes from the small to the large intestine is related to dietary fibre intake and with NSP representing in the order of 35-45 % of the undigested residue. The degradation of the NSP polymers in the large intestine is influenced by factors such as solubility, crystallinity and degree of lignification. NSP have a strong influence on the activity and composition of the commensal microflora, the composition of the short-chain fatty acids produced but it does not seem to have a selective influence on specific strains of microorganisms as has been identified for some NDO (i.e. various fructans). Exogenous enzymes will degrade parts of NSP to mono-, di- and oligomers starting already in the small intestine. In this way the NSP may potentially be used as a substrate to produce oligomers with prebiotic properties. However, convincing in vivo data are still lacking.

**The feed structure** is tightly connected with the feed composition and way the feed is processed. In particular, the fibre content of the raw material plays a role; for example barley produces a more coarse meal than wheat when milled to pass the same screen size. Feeding a coarse diet results in a digesta material that is more coherent, which in turn influences the digestive processes, the microbial ecosystem and the morphology of the gastrointestinal tract. However, the down side of this concept is that, as a result of the coherent properties of coarsely-ground materials, there will be a bigger risk of encapsulation of nutrients, which are then not available for digestion by enzymes in the small intestine.

**Conclusions** The diverse nature of feed carbohydrates makes it possible to influence the gut environment of the pig by choosing among the various feedstuffs and processing conditions.

## What the public want from agriculture: Introduction

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**Introduction** The overall title for these sessions is “Reconnecting the food chain linking farmers scientists and consumers”. Reconnecting the food supply chain was probably the strongest single message to come from the UK Government’s Policy Commission on the Future of Food and Farming (Anon., 2002). Progress on the Policy Commission’s recommendation to establish a Food Chain Centre will no doubt be reported by Dr Segal from DEFRA. The real task of linking farmers, scientists and consumers, however, cannot be accomplished by Government. It is not sensible to expect Government to take on such a role: have we not all got voices?

**The Issues** For the public at large, one of the biggest problems is that there are no issues for many of them – in the sense that much of the public gives no thought to agriculture or to what they want from it. Those who are engaged, however, certainly expect safe, nutritious, healthy food at an acceptable price; a “pleasant” landscape and a “good” environment; access to the countryside. There is also the requirement of a “feel good factor” by many (good animal welfare practices, absence of pollution, biodiversity enhancement, etc) and at least some members of the public would like the agricultural industry to acknowledge the considerable financial contribution it receives from the direct and indirect taxes that the public pays.

**The Links** Reconstructions as envisaged by today’s meeting involves a series of links (each two-way): farmer-public; farmer-scientist; scientist-public. While farmers and scientists have professional bodies involved in these links, and the public has many organisations, the reality is that the majority of these diverse bodies and organisations target links with the Government (and of course attempt to influence Government Policy) rather than each other. This leaves too many of the “links” at the discretion of the media.

Moreover, the national media tends to rely heavily on itself for expertise. Think, for example, of the number of journalists or public relations professionals who have become the media’s scientific and/or environmental “experts”. This is not a criticism of such people. They are excellent communicators and are covering for the majority of scientists who, while good at communicating their science to other scientists, have little or no experience in communicating their science to non-scientists or indeed scientists outside their own discipline.

I suspect that this arises from reductionism in science and the fact that it is often more difficult to do high quality research in areas requiring holistic compared to reductionist approaches. The good news is that most applied scientists have to communicate in order to unearth the next problem to solve. But for how much longer can we continue to leave communications with consumers and the public in the hands of the media, the food processors and of course, the supermarkets ?

The power of the supermarkets in relation to agriculture is twofold. It is not just that they have a high degree of control over agriculture by determining price and volume through their buyers. They are also important as communicators: much of the public’s view of agriculture is conditioned by the supermarkets. Hence, I see the increased interest of farmers in direct sales as important in increasing direct communication between farmers and consumers. The long-term impact of this is likely to be more significant for the industry than merely increasing the proportion of the price consumers pay that farmers receive.

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## **The public and agriculture: the consumer perspective**

Sheila McKechnie,

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Consumers' Association (CA) campaigns on behalf of all UK consumers. CA is a not-for-profit organisation, funded through the sale of our Which? range of consumer information and products. We are founder members of BEUC, the European consumer organisation and Consumers International (CI), the international federation of consumer organisations.

Food has always been one of our main campaigning areas because it is so central to people's lives. We have been key players in debates around food policy over the last twenty years and successfully campaigned for the establishment of the Food Standards Agency, following a succession of food scares and a breakdown in consumer confidence in the way that food issues were handled. The over-arching aim of our food campaign is 'for all consumers to have access to food that is safe, nutritious of good quality and affordable, and to make healthy lifestyle and informed food choices.' Our current campaigns, focusing on areas where we consider there to be significant consumer detriment, include: food labelling and claims, genetically modified foods, prior approval of food premises in order to help reduce food poisoning, and meat safety.

We increasingly have to focus on EU policy in order to ensure improvements for UK consumers, working closely with BEUC - the European consumer organisation - as well as further afield to the body that sets international food standards (Codex Alimentarius) and the World Trade Organisation. We will therefore continue to closely watch the development of the new European Food Safety Authority (EFSA) and ensure that it operates openly and transparently, and effectively involves consumers in its work.

However, one area where consumer concerns are still neglected is in relation to agriculture. The Common Agricultural Policy (CAP) is perhaps unique. In other areas of public policy, citizens pay money as taxpayers to national and local government, revenue which is used to make services – such as medical and dental care, public transport, libraries, leisure facilities, social housing - more readily affordable. But the CAP takes money away from us as taxpayers, and uses it to make the food we buy as consumers more expensive.

EU agricultural policies keep EU food prices higher than prevailing prices elsewhere, and particularly hits low-income families who spend a high proportion of their income on food. The CAP is a substantial misallocation of resources, is inefficient, and is poor value for taxpayers, with a significant level of fraud. The CAP is anti-competitive and distorts markets, and is actually in some sectors a barrier to a single market.

A failure to integrate agriculture within the broader food supply chain has meant that we are still having to deal with a policy that is producer-focused and pays scant attention to the needs and demands of consumers. Following widespread public unease about the safety of food in the wake of the Bovine Spongiform Encephalopathy (BSE), *E coli* and other food scandals including foot and mouth disease (FMD), people are concerned that the large sums of public money spent supporting agriculture are not producing a reliable food supply. The CAP impacts upon the type of food that is produced, how it is produced and how much we pay for it. It distorts demand and imposes a considerable burden - in terms of taxation, higher food prices as well as long-term impacts on public health and the environment.

The starting point for food production has to be to ask consumers what they want and how can these needs can be met. Our concern has been that to date agriculture policy has been mainly focused on producer interests while losing sight of changing consumer attitudes and demands. The irony is that farming can only be successful if it is more responsive to these demands. Sheila McKechnie will explain the findings of CA's research looking at consumer attitudes towards food production.

If we consider, production methods, a similar picture emerges. The public furore over genetically modified (GM) foods has largely resulted from a failure to consider consumer concerns about safety and choice from the outset. This will be a key year as the government decides whether or not to go ahead with commercial planting of GM crops. A public debate has been promised - but its scope and the extent to which it will impact on government policy remains unclear. CA published a policy report at the end of 2002, 'GM dilemmas' which sets out the actions needed in order to refocus the debate on the issues that consumers still want answers to, and considers the implications of products that could be in the pipeline including GM animals and fish.

Some efforts have been made to try and increase consumer confidence in production methods. This includes for example farm assurance schemes. But our research in *Which?* shows that these generally fall short of consumer expectations, often offering little more than the minimum legal requirements.

The Commission's recent proposals for CAP reform, made as part of the Mid-term Review (MTR) of the Agenda 2000 agreement, were an opportunity to address some of these concerns. We would welcome an end to end the link between support for farmers and support for production, but we do not think that the MTR proposals truly achieve this. We want to see any public support linked to food quality, environmental and rural objectives, rather than consolidated payments based on recent levels of support as proposed by the Commission.

We also want to see EU institutional changes, and an end to the domination of agricultural policy by the Commission's Agriculture Directorate-General and EU Farm Ministers at the expense of wider interests.

## **The importance of communication in meeting consumer demands**

Denis Chamberlain, Managing Director, *Chamberlain*

Government, supermarkets, consumers, farming leaders and opinion formers all have a clear view of what primary producers and landowners should deliver in terms of the economic and environmental goods needed to maintain a sustainable, accessible and visually attractive countryside. The trouble is that while each may have a **clear** vision, it is not always the **same** vision.

The key communication chains in the industry are between farmer-market-farmer; between farmer-taxpayer/consumer-farmer; between farmer-scientist-farmer and between farmer-government-farmer.

### **Market**

Signals on quality, farm assurance, price, supply/demand, new product development. Examples of how and where communication is working effectively and where it isn't. For instance, why has trust virtually broken down between farmers (collectively) and supermarkets while many individual relationships thrive to the benefit of both parties?

### **Consumer/taxpayer**

Does farming PR matter or are we too pre-occupied by what people think of us as an industry? Will access legislation begin a healing process between urban critic and rural provider or widen the gap? Are the messages about safety assurance, quality and choice too confused to be read by the average consumer?

### **Scientist**

Farming has built its post-war success on technical and scientific advance but something has gone wrong in the flow of technical information. UK farm productivity and efficiency gains are lagging behind the EU average – as are our farm incomes. Is poor communication at the root of the problem? What can be done to put matters right? Are the post-Curry-Commission recommendations the answer?

### **Government**

Defra has set out its agenda but as agriculture comes to terms with the new rural order (NRO) it is not connecting with government as well as it might. There are too many "old" organisations in the farming industry and in many cases the agenda has been taken over by single-issue political groups. There has been too little co-operation between interested farming parties.

### **Conclusion**

There are now fewer practitioners in active agriculture. Their needs are more specialised and knowledge transfer is not keeping up with the demand. The old arguments about farming's image are redundant. A new level of professionalism is required in communication, extension and reputation management to make sure that all stakeholders in the food and environment chains are working together to improve efficiency and provide a sustainable but productive countryside. Farming must learn to speak the language of the New Rural Order©.

## Physiological genetics, its relationship with classical quantitative and molecular genetics

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Genetic variation in physiological regulation has been studied in humans to explain disposition to hereditary metabolic diseases, such as diabetes, dwarfism and acromegaly. In farm animals the aim of such studies has more often focused on understanding individual variation with a view to develop physiologically based indicator traits that could help speed up genetic improvement programmes. To be efficient, any indicator trait needs to be measurable both early in life and in animals of both sexes. The advent of molecular or DNA based genetic markers has presented us with an alternative set of indicator traits. These may be seen both as competitive traits and as traits, which can be used in combination with physiological indicators.

Synthetic bovine growth hormone (BST) is routinely used in the US to stimulate higher milk yield. However, cows are themselves able to secrete more growth hormone (GH) if they are stimulated by growth hormone releasing hormone (GHRH), and will thereby achieve higher yield. Treatments with BST causes IGF-1 to increase, and this may be an important part in the mechanism of action in the "GH axis". Genetic selection for milk or milk solids yield has been shown to affect plasma GH in cattle, especially following stimulation with GHRH or other secretagogues (Løvendahl et al., 1991; Woolliams et al., 1993; Løvendahl and Sejrsen, 1993). Genetic variation in other parts of the GH-axis is expected, and in pigs plasma IGF-1 concentrations are related to growth rate (Cameron et al., 2001). However, in dairy calves plasma IGF-1 during normal feeding and food deprivation, was not related to dairy merit (Woolliams & Løvendahl, unpublished). As a number of binding proteins interacts with IGF-1 and other elements of the GH-axis, these are not studied in full detail for genetic variance and covariance with milk yield, not least because data has not been available.

The growth hormone gene has a functional polymorphic site giving a substitution between *Leucine* and *Valine* at position 127. The L/V polymorphism has been studied as a genetic marker using a candidate gene approach, for yield (van der Werf et al., 1996). The allele frequency differs between breeds, with Holsteins having low frequency of V compared with Jerseys that have almost equal frequency of the L and V alleles (Sørensen et al., 2002). In Jerseys genotype LL-calves of both sexes released more GH in response to GHRH than VV, with an intermediate response in LV heterozygotes (Sørensen et al., 2002). However, in a larger cohort of Jersey calves this relationship was not confirmed (P Løvendahl & LE Holm, unpublished). The recent findings question the results of the candidate gene approach, at least when a number of modifying factors in the studied axis are remain unknown.

More recently a project aiming at developing physiological predictors has been conducted at DIAS, using future AI-bulls and heifers from the Future Genetics (DK) nucleus herd. Juvenile animals were tested at 9 months of age using a panel of physiological challenges, including a GHRH stimulation test, an adrenaline stimulation test, and a glucose tolerance test. Preliminary results indicate that response variables have intermediate to high heritability (Løvendahl, 2001). From a similarly designed but smaller experiment we have such results. By combining information from into a "physiological index" an accuracy of  $r_{IA} = 0.16$  was obtained in heifers, and  $r_{IA} = 0.07$  in 142 AI-bulls (Sørensen et al., 2000). This accuracy of prediction is comparable to the use of MAS suggested by others (Boichard et al., 2002), and could be applied immediately. By combining MAS and physiological predictors a further improvement in accuracy should be achieved.

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## Organic livestock production in the hills and uplands

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**Introduction** Extensively managed grassland (predominantly hill and upland) represents three quarters of the total land area converted to organic production in the UK. This has occurred because of the availability of conversion aid payments, the downturn in conventional lamb prices towards the end of the 1990's, and a perception that hill and upland farming is already part way towards organic. Organic farming is further encouraged through the Hill Farming Allowance (HFA) scheme, which provides a 10% enhancement for organic production.

**Technical performance** In mixed organic systems, livestock are generally viewed as fertility builders, within a diversity of cropping enterprises. On hill and upland units, sheep and cattle are the main source of income. While hill farming is generally thought to be more 'natural', conversion is not necessarily straightforward. The organic approach emphasises a reduction in external inputs, ideally within a closed system. The natural limitations of hill and upland farming can make this difficult e.g. self-sufficiency in organic feeds. Hill farms have a better opportunity to avoid diseases of intensification such as coccidiosis or E. coli, but trace element deficiencies, tick borne diseases or fluke may be endemic, and require derogation to be sought for specific veterinary intervention. In the more remote areas, practices such as tethering or slatted housing, have been used to compensate for a wetter climate and shortage of straw.

Data on the performance organic systems are available from research at ADAS Redesdale, and it's commercial linked farms, as well as the organic unit at ADAS Pwllpeiran. Overall, the physical and financial implications of conversion, reflect individual farm circumstances. Choice of stocking rate, relative to the production potential and specific limitations of the farm, is critical in determining performance. For an extensive hill farm, selling store lambs at weaning, little adjustment may be required. The implications will be greater for an upland unit carrying sheep and cattle at higher stocking rates, and home-finishing the majority of stock produced. A well managed, clover-rich upland sward is capable of producing 80% the dry matter output of a highly fertilised pasture. Individual animal performance may also improve, even in ensiled material (Keatinge and Murray, 1994). However, pushing stocking rates towards conventional levels in pursuit of maximum output, will compromise individual animal performance (Keatinge, 2001), and ultimately will not be sustainable. Mean stocking rates on the commercial organic farms linked to the Redesdale Unit, are 1.0 Grazing Livestock Units (GLU) per hectare – comparable with Newcastle University (0.7 GLU/ha) and University College Wales (1.3 GLU/ha) conventional Farm Business Survey farms respectively. At Redesdale, typical growth rates to weaning from pure-bred Scottish Blackface lambs are 200 – 230 g/day. Conception rate (95%), calf growth rate to weaning (1.0 kg/day) and from birth to sale (0.8 kg/day) are within expected ranges for a spring calving suckler herd. During the late 1990's, organic lamb prices were up to 40% above those of conventional. As supplies increased, prices have fallen and a significant proportion is again being sold on the conventional market. While the sale of store stock is very compatible with hill production, finishing capacity needs to be expanded on lowland arable farms. Neither are producers immune to increasing imports, currently running at approximately 30% for organic beef.

Environmental benefit is one of the main political drivers for organic farming. Evidence for the environmental benefit of organic farming *per se* is less clear than in the lowlands (Adamson et al, 2002), and a more proactive approach is required. Although widely used by organic farmers, agri-environmental schemes such as Countryside Stewardship or ESA Schemes are not always compatible with organic farming practice, or regulation. If stocking rates must be reduced to accommodate a more sustainable system, a combination of better integration with agri-environmental schemes, reduced input costs and modest price premia, will be required for economic viability.

**Conclusion** It is likely that organic production will remain a significant factor in hill and upland farming. Despite the challenges, it continues to be supported politically, and is consistent with objectives for reducing stocking pressure, adding value, regional or local market identity. Although long-term economic viability has yet to be proven, the greater the extent to which agricultural support is decoupled from production, the lower the potential costs of organic conversion.

**Acknowledgements** DEFRA funding for the organic research at ADAS Redesdale is gratefully acknowledged.

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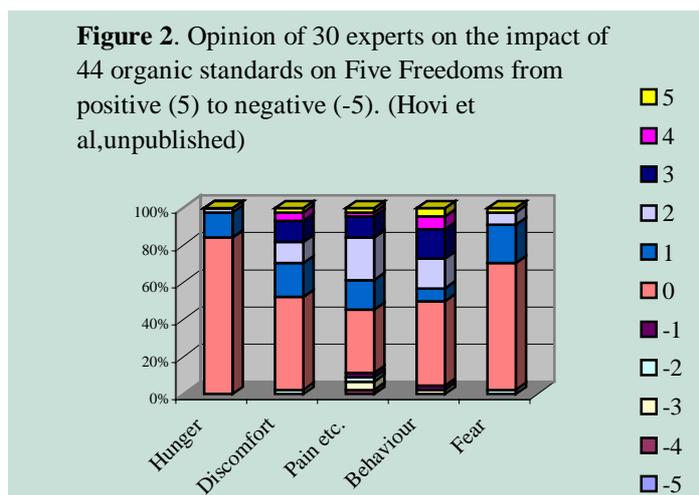
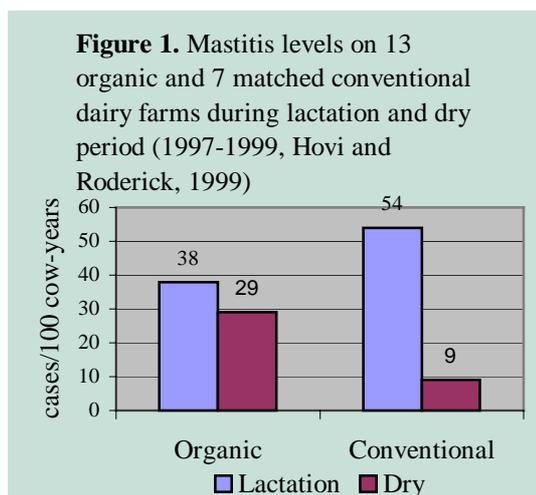
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## Animal health and welfare in organic livestock production – a review of the current situation

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**Findings** According to the EC Regulation 1804/99, health and welfare of organic livestock should be promoted primarily by preventive measures using appropriate breeds, feeds and feeding practices and husbandry techniques for the species in question and by implementing stable social conditions for breeding animals (CEC, 1999). Whilst the EC Regulation on organic livestock production came into force fairly recently in August 2000 and has hardly had a chance to have an impact on existing organic production systems, there is a growing body of epidemiological evidence on the impact of organic management on the health and welfare of livestock. Furthermore, a three-year networking project, the Network for Animal Health and Welfare in Organic Agriculture (NAHWOA), between 17 institutes from 13 different EU countries has recently published their conclusions and recommendations on animal health and welfare in organic production systems (Anon, 2002). A recent review of literature (Hovi *et al.*, in press) and the NAHWOA conclusions suggest that animal health situation in organic livestock systems is similar to that found in conventional systems. Some differences in the prevalence of different conditions exist. A typical example of higher dry period but lower lactation period levels of mastitis in organic than in conventional systems is presented in Figure 1. It has been suggested that the minimal organic standards and their implementation via certification procedure are likely to provide several preconditions for good living conditions for farm animals (Sundrum, 2001), and the NAHWOA concludes that the current evidence supports the claim that organic livestock production often provides better welfare than conventional production systems, particularly by providing more freedom for species-specific behaviour for livestock. It has, however, been suggested that the organic standards do not necessarily provide a balanced approach to animal welfare and that some conflicts between welfare aims and other organic farming objectives may exist (Anon, 2002). An example of how experts perceive the impact of organic standards on animal welfare is given in Figure 2.



### Conclusions and future challenges

There is a need to ensure an improvement in animal health situation in organic livestock production. It is envisaged that this would best be achieved, within the standards and objectives of organic farm assurance, by ensuring the implementation of useful and transparent animal health planning on farms and by developing breeding strategies that allow individual farms to establish herds/flocks that are suited to the local conditions and can maintain high health status under the particular husbandry system. There is also a need to identify the areas where organic production objectives may conflict with the objective of maintaining high animal welfare and to establish safeguards that both prevent animal welfare breaches from happening and maintain the integrity and consumer confidence of the assurance programme.

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## Control of endoparasites in organic livestock systems

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**Introduction** Endoparasite control in organic and non organic livestock shares many features. The difference is that organic farmers are constrained by the requirements of the standards which regulate organic production (UKROFS, 2001). This paper reviews the alternative strategies currently available.

**Organic standards** Animal health in organic livestock production is based on disease prevention without the routine use of veterinary medicines. This is to be achieved by the application of sound management practices; breed and strain selection; preventive husbandry; optimal nutrition and appropriate stocking densities. The standards prefer the use of herbal or homeopathic medicines where these can be shown to be efficacious. Conventional veterinary medicines may not be used to prevent disease but can be used to treat clinical cases. However, the strategic use of medicines is permitted where an identified disease risk cannot be prevented by management practices alone. The animal health strategy must be formalised by the production of an animal health plan specific to the individual farm.

**Principles of control** Most endoparasites are endemic in the British Isles, although they show regional variation in incidence. Eradication from individual farms is not an option. Instead control seeks to limit exposure of susceptible animals to parasites and to improve the ability of animals to develop immunity following exposure. Organic systems require access to pasture for ruminant and monogastric animals. Grazing management thus provides a mechanism by which parasite challenge to individual animals can be reduced. A knowledge of the epidemiology of the specific parasite is required if practical means of control are to be developed. Immunity to parasites is modulated by genetic and nutritional factors as well as by exposure. Novel forage crops such as Lotus or chicory are being evaluated for their parasite control properties. Genetic selection for resilience or immunity to parasites is also being studied.

**Practical control measures** Few organic farms achieve parasite control without some medicine inputs (Roderick and Hovi, 1999). An integrated parasite control strategy should be the aim. This combines grazing management, nutritional and breed aspects with strategic dosing where necessary. Clean grazing systems for ruminants are well known but integration of other animal species such as pigs and poultry into organic rotations is also practised. These systems are based on the host specificity of most endoparasites but may break down when parasites such as *Nematodirus battus* and *Trichostrongylus axei* are able to infect more than one animal host. Variations on classical clean grazing include alternate grazing between species such as cattle and sheep on an annual basis or mixed species grazing with cattle and sheep or cattle and pigs sharing the same pasture. Mixed grazing results in reduced exposure to parasites as a result of host specificity and reduction in effective stocking density. Annual rotational systems give good endoparasite control in cattle but the periparturient rise in faecal worm egg output by ewes can result in significant parasitism in lambs in late summer (Gray, 2002). In such situations, strategic worming of ewes at or after lambing may be needed to prevent pasture contamination and avoid clinical disease in lambs. Due to seasonal variability, organic farmers are being encouraged to monitor the endoparasite control situation on their farms. This is most easily done by carrying out strategic faecal worm egg counts on groups of susceptible animals such as lambs. This "contamination mapping" can be done by the farmer using commercially available kits or by veterinary laboratories.

**Problematic areas** In areas where climate and soil type permit, the integration of animals into a varied cropping and grazing rotation can be very successful. In such cases, the need for anthelmintics can be minimised. However, organic systems in less favoured areas can be problematic. The extreme example is sheep only hill and upland farms where grazing management options are limited. Epidemiological considerations mean that the control of liver fluke and lungworm is difficult to achieve by husbandry measures alone. Strategic dosing for fluke and vaccination against lungworm are used frequently.

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## Managing manures in organic farming

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**Introduction** Knowledge of manure composition is important for farm nutrient management, either if importing manure onto a farm or transferring nutrients around the farm in 'home produced' manures. Many factors affect the nutrient content of the manure ready to spread onto the land (Smith & Frost, 2000): dietary input and quality, nutrient losses during housing and storage and additions of bedding material and/or water. There are many reports of average values for manures from conventionally raised livestock (e.g. Anon., 2000 for the U.K.). However, there are less data available for manures produced on organic holdings. It is probable that composition will differ from conventionally produced manures because of differences in diet and manure storage methods (composting and/or long-term storage). Therefore, we aimed to test this under U.K. conditions by analysing cattle manures from organic holdings for comparison with data on conventionally produced manures.

**Materials and methods** The study focused on cattle manures because this is currently the largest organic livestock sector in the U.K. Twenty-nine organic farms were visited and representative samples (c. 3 kg) of FYM or slurry were collected from the stores. Forty-three FYM samples were collected. Fewer slurry samples were collected (14), reflecting the greater reliance on straw-based animal systems. The manure nutrient content was determined by standard analytical techniques (Anon., 1986).

**Results** The slurry samples showed a wide range of dry matter contents and nutrient values, characterised by large standard deviations about the mean. Nutrient concentration in fresh slurry was strongly correlated with slurry dry matter content, although K concentration showed the weakest relationship (Table 1). This is because K is predominantly associated with the urine, rather than the solids fraction. The data set of 'conventional' manures that underpinned the

**Table 1** Regression lines fitted to nutrient (kg/m<sup>3</sup>) and dry matter (%) contents for slurry samples (n = 14).

Regression equation	r <sup>2</sup> (%)	P value
N = 0.35 + 0.27 DM	61	< 0.001
P = 0.07 + 0.04 DM	71	< 0.001
K = 0.83 + 0.16 DM	30	0.025
Mg = 0.06 + 0.03 DM	65	< 0.001
S = 0.02 + 0.03 DM	75	< 0.001

U.K. fertiliser recommendations (Anon., 2000) was then compared with the new, organic data by parallel curve fitting. For the three variates tested (total N, P and K: no data for S or Mg for conventional slurries), it was found that there was a significant improvement by fitting parallel lines to the two sets of data, but that there was no additional significant improvement in allowing the slopes to vary. This suggests that, for example, the rate of increase in total N concentration with increasing dry matter is not significantly different in the organic and conventional slurries, but that the conventional slurries start at a higher initial level. The intercept of the regression of nutrient content and dry matter represents the concentration in the liquid phase only (i.e. 0% dry

matter). These intercept values for N, P and K, respectively, were (conventional, organic): 1.26, 0.20; 0.16, -0.09; 1.44, 0.84 kg/m<sup>3</sup>. This therefore clearly shows that, on average, the N, P and K contents of conventionally produced slurries is greater than those from organic holdings. Comparisons were also made with the U.K. available data on conventional FYM and FYM from organic holdings. There were no statistically significant differences between data sets, but variability around each mean was large. Based on findings for the slurry samples, which are effectively diluted urine and faeces, it is likely that the excreta contributing to the FYM also contained smaller concentrations of nutrient than would have been the case on conventional holdings, though this could not be proven. However, many nutrient transformations occur, including losses, in FYM between excretion and removal from the store. It may be that any differences at the start may be lessened by the time the FYM leaves storage.

**Conclusions** Manures play a key role in fertility building and maintenance in many organic rotations. Understanding their nutrient composition and nutrient availability is therefore important for optimising their use on farm. The measurements show that cattle manures from organic holdings can have slightly lower nutrient contents than their conventional equivalents, but variability is large. Therefore, much of what we know about managing conventional manures can be adapted to organic agriculture. Avoiding autumn applications of slurry reduces nitrate leaching loss; rapid incorporation will minimise ammonia loss, for example.

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## **Emerging animal welfare standards: science, values and public trust**

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During the last decade, many of the developed countries have seen a rapid move toward explicit farm animal welfare standards. Three historical developments during the last half century have contributed to this move: (1) the emergence of intensive animal production systems developed on the principle of maximizing production efficiency, (2) the perception (sometimes justified, sometimes not) that animal production is becoming an industrial, technological activity substantially controlled by corporate owners, and (3) a rapid increase in humanitarian attitudes toward animals.

These changes have also contributed to three different views of how animals should be raised and, hence, how animal welfare should be assessed. One is a “biological functioning” view which holds that animal welfare depends on a high level of health, growth, production efficiency and correlated traits; this view is especially common among intensive animal producers and some veterinarians and animal scientists. A second is that animals should be allowed to lead relatively natural lives and use their species-typical adaptations. This view is common among consumers and many social critics of modern animal production. A third view emphasizes the “affective states” of animals, with emphasis on preventing negative states (pain, suffering) and permitting positive states (comfort, contentment). This view is common in humanitarian thinking and among some animal welfare scientists. Scientists have claimed scientific validity for all three of these views, sometimes also claiming that the other views lack scientific validity.

Each of these views has influenced animal welfare standards. Some widely followed standards of animal housing, such as those of major chain restaurants in the United States, are based largely on biological functioning criteria. For example, these set space allowances based on maximizing production variables such as survival, rate of lay, or rate of gain. Standards in organic and some alternative production systems are based more on a natural living approach, requiring space and amenities that allow animals to perform key elements of their natural behaviour. Certain other standards, for example in humane slaughter, are based mainly on affective state criteria, seeking to prevent pain, fear and distress.

With a wide variety of animal welfare standards being promoted, attention will be needed to three factors if public trust in the process is to be maintained. (1) With conflicting standards claiming to indicate a high level of animal welfare, there is a need for clear communication about the basis on which the standards have been developed, and for honest description of the trade-offs among different elements of animal welfare. (2) Given the diversity of views of animal welfare extant in the public, standards are not likely to maintain public trust if they focus on only some aspects of animal welfare and ignore others. For example, standards based on natural living criteria will not maintain public trust if they provide inadequate protection of basic animal health, nor standards based on biological functioning criteria if they take no account of natural behaviour or affective states. (3) Given the widespread perception that food safety is related to animal welfare, food safety considerations will need to be part of animal welfare assurance systems if they are to retain public trust. [bsas2003]

# Invited Papers

(not available at time of Proceedings going to press)

## ANIMAL SCIENCE AND GLOBALISATION

- 243 The Livestock revolution/implications – an African view  
*D. Miano Mwangi<sup>1</sup> and A. Omore<sup>1,2</sup>*  
<sup>1</sup>*Kenya Agricultural Research Institute P.O. Box 30148 Nairobi, Kenya*  
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## NUTRITION AND ENVIRONMENTAL IMPACT

- 245 Options for change - Reducing environmental emissions from livestock production.  
*S.C. Jarvis, IGER, North Wyke Research Station, Okehampton, UK*
- 246 Reducing methane production from ruminants – a practical approach  
*F.P. O'Mara and D.K. Lovett, University College Dublin, Ireland.*

## AQUACULTURE

- 248 The Industry, European and Global aspects  
*K Rana and K Jauncey, Institute of Aquaculture, University of Stirling, Stirling, UK*

## RE-CONNECTING THE FOOD CHAIN - LINKING FARMERS, SCIENTISTS AND CONSUMERS

### SESSION 1: WHAT THE PUBLIC WANTS FROM AGRICULTURE

- 249 What the public wants from agriculture: The government view  
*M Segal, Head Of Livestock Strategy, DEFRA*

## **The Livestock revolution/implications – an African view**

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### **Introduction**

The rapid increase in the production and consumption of livestock and livestock products fuelled by population growth, urbanisation and increase in average per capita income has come to be known as the livestock revolution (Delgado *et al* 1999). A rapid growth in per capita consumption of livestock products in developing countries over the last decade (FOASTAT, 2002)

If the Africa has to meet the demand for livestock and livestock products driven by the livestock revolution and reduce the need to import these products several constraint to production must be addressed. These include;

- Intensify production
- Build institutional and infrastructural capacities
- Remove policy distortion that curtail livestock development
- Livestock diseases
- Environment and animal welfare

### **Intensification and diversification of the production system**

In order to meet the increased demand for livestock and livestock products the production systems in Africa must change. Intensification of production systems has been taking place in Africa given the limited scope of extending the area and number of animals. Issues on breeds used and the feed resource must be addressed. Considering that mixed crop-livestock systems predominate in Africa (McIntire, 1992), the intensification process will have to address both the crop and livestock components of the production as a shift to a more specialised production system is not envisaged. The Kenya dairy industry is a good example for intensification. The upgrading of the local animal with exotic dairy breeds has been a major success in the intensification process. This has been accompanied by more intensive use of the land and as the land available for pasture production declined the cut-and-carry system developed where high yielding fodder crops including napier grass (*Pennisetum purpureum*) have replaced pasture (Staal *et al.* 2002). Due to this process the smallholder dairy farmers, mainly family farms with an average of 2-3 animals produce approximately 80% of the marketed milk in Kenya.

Livestock keepers in Africa do not keep animals solely for economic reasons (generation of income and employment) but also for manure and traction power. In crop-livestock systems manure is a very important product as it is vital for crop production. Although manure will not help produce all the food that Africa requires, combining them with small amounts of organic fertiliser does not only results in higher yields but more sustainable production. In Niger, tethering of animals on cropland for 3 days increases the pearl millet yields to about 180 kg in a plot 4 x 4 m compared to 10 kg when no manure was applied (ILRI 1997). Mixed farming also allows the poor livestock farmers to convert the poor quality crop residues to valuable livestock products.

### **Development of infrastructure**

Smallholder farmers in rural areas produce most of the livestock and livestock products in Africa in mixed farms. Most of the livestock and livestock products are consumed in urban areas for example 60% of the marketed milk in Kenya is consumed in Nairobi. The products have to be moved from producing areas to consumption areas. Poor infrastructure has inhibited the ability of livestock keepers in Africa to market livestock and livestock products. In Kenya it is has been shown that for every kilometre of bad road a dairy farmer losses 0.34 cents per litre of milk traded, the losses could be higher in beef animals which have to trek long distances to the nearest abattoir. Africa will need assistance from the developed world to develop infrastructure required in order to improve production and quality of livestock and livestock products for local consumption and export.

### **Policy distortions**

Kenya with one of the most developed dairy sectors in Africa, has adopted a cold chain model used in developed countries. The policies governing the dairy industry are therefore geared towards this model. This is despite the fact that only about 12% of the marketed milk passes through this formal system. Informal milk traders handle 38% of the marketed milk (Anderson *et al.* 2002). It has also been shown that every litre of milk handled through the small milk traders creates more jobs than that through small processors (Staal *et al.*, 2002). Yet for Kenya to be able to export milk in the region processing capacity must be improved. Therefore, policies that will integrate the informal traders into the mainstream milk marketing equation are necessary.

Developed countries especially EU and North America use subsidies and other trade barriers to protect domestic. These subsidies have distorted the patterns of international trade and although the developing countries hold a large proportion of the world's livestock, they contribute only a small proportion of the world trade in livestock and livestock products. Although, WTO aims at reducing distortions caused by subsidies, the introduction of non-tariff barriers will continue influencing trade in livestock products. International standards when embedded into national standards will also affect cross-border regional trade. Therefore, the raising of standards can lead to public services and markets to discriminate against poor livestock keepers. The other main problem is that the poor nations of Africa do not effectively participate in setting the standards and methods that improve their participation in setting appropriate trade standards should be developed (Holden, 2002).

### **Livestock diseases**

Livestock diseases do not only reduce productivity but also has major implications in the potential of Africa to export livestock products. The sanitary and phytosanitary (SPS) measures imposed by the developed world will curtail export of livestock products from Africa. For Africa to be able to comply with the SPS measures it will require both technical and financial support from the developed world.

### **Environment and animal welfare**

In the urban and peri-urban areas of Africa, poultry and pig production systems that require less land are increasing rapidly due to the demand of these products by the urban population. These systems are associated with difficulties in disposing manure, leading to pollution. Issues of animal welfare are also starting to emerge. Policies need to be developed to address these issues.

### **Conclusion**

Africa has remained a net importer of livestock products (FAOSTAT 2002) and with the projected increased demand the continent might remain a net importer for sometime. Importation of cheap livestock products might benefit the consumers who are mainly the urban rich and disadvantage the mainly rural poor livestock producers. On the other hand policies that encourage trade would have a major impact on poverty in the continent as majority of livestock producers are in the poor strata of the society. Due to the trade barriers imposed by the developing countries Africa will not be able increase her participation in international trade unless the developed countries help in development of infrastructure and disease control.

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## **Options for change - Reducing environmental emissions from livestock production.**

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Livestock production is inherently 'leaky' with materials moving from the farming system into waters and the atmosphere as part of the biogeochemical cycling processes that occur in all ecosystems. Increasingly, there are national and international legislative pressures to minimise fluxes of environmentally active materials into waters and the atmosphere. Public opinion is also demanding that the husbandry and management of livestock production is adjusted to take account of the short and the long term impacts on environmental quality. Although livestock farms may be responsible for point source effects these have diminished over recent years and the greater concern is with diffuse pollution effects.

Diffuse pollution generally comes about because of the low efficiency of utilisation of the nutrients that are required to sustain production under current conditions. The resultant flows and distribution of excess carbon, nitrogen and phosphorus create opportunity for the escape of these nutrients into the wider environment, generating enhanced concentrations of  $\text{NO}_3^-$ , P,  $\text{CH}_4$ ,  $\text{NH}_3$ ,  $\text{N}_2\text{O}$  as well as other materials in circumstances where they may act as pollutants.

Research has done much to identify and quantify the extent of the emissions from various livestock systems, and, at least in part, to define some of the controlling variables. In many cases, models of varying sophistication have been developed to aid prediction and decision making. The current need is to provide guidance for policy makers and land/farm managers to promote and employ mitigation options that not only reduce the particular problem but also allow sustainable production. Much current research is directed at providing effective solutions for this. The problems are confounded by the significant and far-reaching changes that are occurring currently within the agricultural sector. International trade agreements, changes in EU policies (and composition), polarisation of the agricultural industry, and the growing need to consider agriculture as an integral part of a rural landscape rather than single component units will all impact on how problems are dealt with in the future.

Livestock farms are inevitably 'leaky' but there are opportunities to reduce impact. Some of these may not be easily implemented especially against a backcloth of substantial change. However, it is clear that the continuing discharge of excessive amounts of diffuse pollutants which can be generated by livestock production will not be acceptable and practices will have to be put into place to minimise these. A primary need will be to define what actually happens on farms which are the functional units within which changes have to be imposed. Such definition will enable benchmarking to be made and indicators developed to identify further needs and to monitor change. There is also a need for an integrated systematic and multifunctional approach so that 'pollution swapping' does not become an issue not only for direct on-farm effects, but also for those which are developed further 'downstream' and at a wider range of scales. This paper provides a discussion on options to for ways forward.

## Reducing methane production from ruminants – a practical approach

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**Introduction** Although methane production from enteric fermentation in ruminants has been studied for many years, it is only recently that research has focused on reducing methane production in order to reduce greenhouse gas emissions. Many new and novel strategies are being researched, but this paper focuses on mitigation strategies that could currently be implemented from the knowledge already available.

**Increased animal productivity** Increasing animal productivity will generally reduce methane emissions per kg of product (milk or meat). However, annual emissions per animal per year are usually increased because the higher productivity is usually associated with higher dry matter (DM) intake. This has a different impact on the dairy and beef sectors. In the dairy sector, increasing productivity will probably increase total emissions within a country, as producers are unlikely to reduce animal numbers unless there is a ceiling on production as in the EU. Kirchgessner *et al.* (1995) reported that increasing milk yield from 4000 to 5000 kg/year increased annual methane emissions, but decreased emissions per kg of milk by 0.16, whereas an increase from 4000 to 6000 kg/year would decrease emissions per kg of milk by 0.29. In the beef sector, increasing productivity results in animals reaching a target or acceptable slaughter weight at a younger age, and this can have a major impact on lifetime emissions. Lovett and O'Mara (2002) calculated that reducing age at slaughter from 30 to 25 months resulted in a 0.165 reduction in lifetime methane emissions and a 0.12 reduction in emissions per kg of carcass (due to a reduction in carcass weight from 400 to 380 kg).

Intensive production systems which result in lower slaughter weights such as 'barley beef' or young bull production could lead to dramatic reductions in lifetime methane emissions compared to conventional steer production systems because of the reduced lifetime DM intake, and the high concentrate proportion in the dietary DM causing a low conversion to methane. In 'barley beef' production systems, calves are conventionally reared to about 3 months of age, and they are then placed on ad libitum concentrate diets until slaughter about nine months later at carcass weights of approximately 250 kg. Typical concentrate intake is about 7.4 kg/d (Keane, 2001). Because concentrates constitute about 0.9 of dietary DM, methane emissions are low. Using a figure of 0.04 of gross energy intake (GEI), total lifetime emissions are 27 kg, in comparison to over 100 kg for conventional steer production systems (Lovett and O'Mara, 2002). In bull beef production, weanlings that have spent their first summer at grass are housed and fed ad libitum concentrates for 6 to 9 months and then slaughtered. Typically for a six month feeding period, concentrate DM intake averages 8.5 kg/d and the resulting carcasses are about 300 kg (Keane, 2001). During this period, calculated methane emissions are 23 kg (because concentrates constitute about 0.9 of dietary DM, methane emissions are calculated as 0.04 of GEI) which is substantially lower than conventional 24 month steer production systems.

Focusing on improving productivity in the finishing period, we analysed the effect of increasing concentrate level on methane emissions over the period required to go from 550 to 650 kg liveweight for a Charolais × Friesian steer. Three concentrate levels were examined: 3 kg/d, 6 kg/d and ad libitum concentrates. The model of Yan *et al.* (2000) was used to estimate methane output, except for ad libitum concentrate diets (0.04 of GEI). Increasing the concentrate level from 6 kg/d to ad libitum lowered methane emissions by 0.41, whereas decreasing the concentrate level from 6 to 3 kg/d led to an increase in total methane emissions of 0.36. The decrease resulting from increased concentrate feeding is a result of both a reduced feeding period and a reduction in the proportion of GEI emitted as methane.

**Increasing the concentrate proportion in the diet** Generally, as forage is replaced by concentrates, DM intake increases and animal production increases. With regard to methane energy output when expressed as a proportion of gross energy intake, a curvilinear response has been widely observed (i.e. methane output increases as concentrate level is increased from low to medium levels, but further increases in concentrate feeding lead to reductions in methane output). The increases in methane output at low levels of supplementation arise due to an increase in the amount of substrate fermented within the rumen. The observed decrease in emissions at high levels of supplementation is explainable firstly through an increase in the rate and amount of propionate production (a ruminal H<sub>2</sub> sink) but also because of a reduction in the pH of the ruminal environment, as methanogenic bacteria are acutely pH sensitive. Increasing the concentrate proportion of the diet will also have an effect on animal productivity be it dairy or beef animals with the result that emissions per unit of animal product are reduced as outlined above.

This effect could also be exploited by changing the pattern of allocating a fixed amount of concentrates to finishing cattle. Keane (2001) has investigated the effect of pattern of concentrate allocation on animal performance. Performance was similar for animals fed a fixed level of concentrates throughout the finishing period as for animals fed the same target total concentrate allowance with none for the first half, but ad libitum for the second half. Using the model of Yan *et al.* (2000) to predict methane emissions of animals except for ad libitum concentrate diets (0.04 of GEI), methane emissions would be reduced by 0.11 by adopting the stepped concentrate regime.

**Concentrate composition** Changing the composition of concentrates has been shown to impact on methane emissions. Structural carbohydrates (cellulose and hemicellulose) ferment at slower rates than non-structural carbohydrates (starch and sugars) and yield more methane per unit of substrate fermented due to a greater acetate:propionate ratio (Czerkawski,

1969). It has also been suggested that non-structural carbohydrates should be further subdivided as soluble sugars have a higher methanogenic potential than starch (Johnson and Johnson, 1995). Modelling exercises have demonstrated that replacing fibre (Benchaar *et al.*, 2001) and sugars (Mills *et al.*, 2001) within concentrates will reduce methane emissions arising from enteric fermentation. Benchaar *et al.* (2001) also demonstrated that substituting rapidly degradable starch for more slowly ruminally degradable starch (e.g. maize for barley) will lead to a further reduction in methane output, as relative to barley, maize tends to produce a reduced acetate:propionate ratio.

**Addition of oils to the diet** Machmuller and Kreuzer (1999) demonstrated that coconut oil was effective in reducing methane emissions. Coconut oil is high in lauric and myristic acids and these have been repeatedly demonstrated to have strong anti-microbial properties on both protozoa and methanogenic bacteria. Recent research at our institute (Lovell, 2002) has demonstrated that coconut oil fed to finishing beef heifers at a level of c. 5% of DMI can cause a reduction in methane emissions of up to 0.33, without any significant negative effect on animal performance. However, effects on DM intake and fatty acid composition of sub-cutaneous fat need further research, as there was a negative effect on DM intake and a positive effect on sub-cutaneous fat concentrations of lauric and myristic acids due to coconut oil supplementation in this study.

**Basal diet** Typically in Ireland grass forms the main dietary component, be it at pasture or following conservation as silage, for over-wintering animals and those entering a finishing system. Substituting grass as the main dietary component for ruminants offers a means to reduce enteric methane emissions. Studies have demonstrated that increasing the legume content of pasture results in reduced methane emissions presumably due to higher digestibility, modified fermentation patterns and increased passage rates. Also there is a considerable body of evidence demonstrating improvements in animal performance in terms of intake and animal productivity through the substitution of grass silage by maize silage when animals are housed and in addition reductions in methane output as a proportion of gross energy intake are to be expected due to a reduced acetate:propionate ratio in ruminal volatile fatty acids.

**Conclusion** There are a number of options currently available to producers to reduce methane emissions. The most dramatic reductions will come from improved animal productivity. This can be achieved through animal breeding, nutrition and management. Additionally, there are some dietary strategies that are at or near the application stage that could be used to reduce methane emissions without any significant impact on animal performance.

**Acknowledgement** The authors acknowledge the support of the Environmental RTDI Programme 2000-2006, financed by the Irish Government under the National Development Plan and administered on behalf of the Department of the Environment and Local Government by the Environmental Protection Agency.

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## The Industry, European and Global aspects

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### Introduction

Aquaculture, the farming of aquatic organisms, is amongst the few animal food production sectors that has continued to show strong growth over the last 30 years and over the last decade achieved an annual increase of 7.5% compared with 2.5% for meat production. This blue revolution perhaps marks the last phase in animal domestication and the prognosis for sustainable growth of the global sector is good. In 2000 around 45.7 million tonnes of aquatic produce valued at US\$ 56.5 billion was produced.

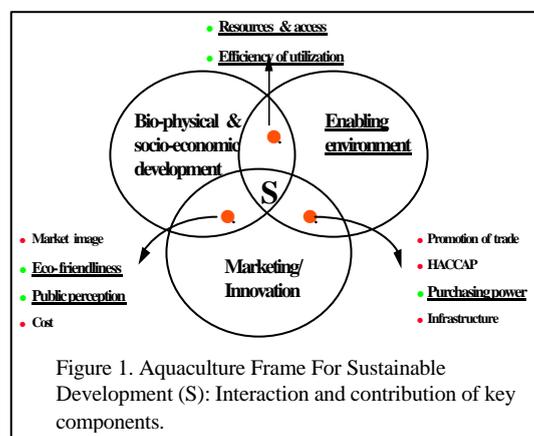
### Trends in Global Aquaculture Production

Unlike terrestrial animal production there is a high utilisation of aquatic biodiversity in aquafarming. In 2000, FAO Member countries reported the culture of over 180 of aquatic organisms with a production of over 500 tonnes. Contrary to common perception, aquatic plants and filter feeding invertebrates, low in the food chain, account for close to half the global production. Similarly, in Europe filter feeders such as molluscs account for nearly 40% of aquaculture output. Asia continues to dominate global aquaculture output, accounting for 91% production by quantity with Europe accounting for only 4%. These trends and key global and regional features of production, including those species groups that are traded as commodities will be discussed.

### Key issues facing aquaculture development and trade

The domestic and international demand for both high and low valued aquatic products is forecasted to increase due to a combination of rising populations, living standards and disposable incomes. It is widely acknowledged, that fish yield from **traditional** capture fisheries, however is unlikely to increase substantially to meet future needs (SOFIA, 2000). Much of this demand is expected to be met from aquaculture. The challenges the sector therefore face are how to: (i) sustain and increase the current mean annual global growth rate and (ii) strengthen and promote aquaculture<sup>1</sup> as a legitimate long term farming activity. The extent to which these challenges are addressed vary between nations depending primarily on the state of aquaculture development and its economic importance.

Sustainable aquaculture development is influenced by external and internal factors (enabling environment) and therefore the probability of achieving a sustainable industry is likely to be realized through practical regulations within the broader national and regional framework of land and water utilization. Sustainable aquaculture development at the national and enterprise level depends on the extent to which each of the several inter-dependant components successfully co-ordinates, contributes and interacts to support the other (Figure 1).



In Europe the future direction of aquaculture is being influenced by stricter environmental regulations, market saturation of mainstay fish species such as salmon, trout, sea bass and bream and the search for new species, bio-security arising from the spread of disease, food quality, tracability and labeling. Crucial current issues facing developing countries trading in farmed fish commodities are trade barriers whereas nationally, countries are attempting to develop regulatory framework aimed at increasing sustainable expansion and improving production efficiency.

### Conclusion

Fish consumption has increased globally but future demands will have to be met largely from aquaculture. Although great strides have been made in engineering and biological technologies in the last three decades, a sustainable aquaculture industry, can probably only operate within a national regulatory framework that strongly supports the sector.

## **What the public wants from agriculture**

### **The government view**

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The public's relationship with agriculture is complex and multi-faceted. Agriculture not only produces a large proportion of the food we eat, but is also responsible for maintaining the majority of the landscape and countryside. The public are customers for both of these activities and for a variety of other services provided by farmers, whether in the narrow sense of paying directly for them or, more widely, as users or benefiting indirectly. The Curry report stressed the need for farmers to reconnect both to the environment and to their customers. Given that the theme of this conference is reconnecting the food chain, this presentation will concentrate mostly on the latter, but I will demonstrate that the sustainability of the farming industry depends critically on getting all these aspects right.

In this presentation I shall discuss how it is possible for us to know what the public want: from surveys, from their purchasing patterns and from their general behaviours. The evidence is often inconclusive or contradictory. Nevertheless, it underpins Government policy and I shall describe our approach to the future of the industry, as outlined in the Strategy for Sustainable Farming and Food. Reform of the CAP is a key objective, since existing production-linked subsidies are the source of many of the problems. However, CAP reform alone will not deliver everything the public wants; equally, there is much we can do even if the current mid-term review of the CAP fails to deliver fundamental reform. I will give examples of the initiatives that are already underway.